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PARASITISM, MORPHOLOGY, AND CYTOLOGY OF CRONARTIUM RIBICOLA

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INTRODUCTION

During the last few years *Cronartium ribicola* Fischer has become the most serious fungus pest of white pine (*Pinus strobus* L.) in America. As such it has been the subject of much study by pathologists who realized, from a knowledge of the course of the parasite in Europe, the damage it was capable of causing. Spaulding (*54*)[1] in the most comprehensive paper on the fungus which has appeared in this country, reviews the reports of its ravages on white pine in the Old World and gives its general characters and life history. Since the discovery by Stewart (*57*) of *C. ribicola* on species of Ribes at Geneva, N. Y., in 1906, many papers have appeared calling attention to the absolute necessity of controlling the spread of this parasite if the white pine is to be saved for reforestation. A great deal of attention has been given to testing the susceptibility of possible hosts through inoculation and to control and eradication methods, both in the United States and Canada. In Europe most of the recent work has been along this same line. Klebahn (*23*, *24*, *25*, *26*) and Tubeuf (*58*, *59*, *60*) have carried on extensive experiments.

The morphology of *Cronartium ribicola* and the details of the interrelations of the parasite and its hosts have never been thoroughly worked out. The cytology of the genus Cronartium has up to the present time received very little attention. In the following paper the results of certain observations extending over a period of two years will be presented, first, with reference to the minute histology of the fungus and the interrelations of host and parasite, and second, with reference to the cytological phenomena accompanying spore production in the different types of sori. The paper is offered as a contribution to our knowledge of the parasitism, morphology, and cytology of the genus Cronartium.

[1] Reference is made by number (italic) to "Literature cited," p. 655–659.

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LIFE HISTORY AND HOSTS

The life history of *Cronartium ribicola* has been so well reported by Tubeuf (*59*, *60*), Klebahn (*25*), Spaulding (*54*, *55*), and others that it is only necessary to repeat it in outline by way of introduction to what follows. Sporidia from the teliospores produced on *Ribes* spp. infect young stems and branches of *Pinus strobus* and other 5-needled pines. The pycnial and æcial stages subsequently develop on the pines. Æciospores from the pine infect leaves of *Ribes* spp., on which the uredinia are shortly formed. These sori are produced in successive generations throughout the summer. Telia develop from old uredinia, or as separate entities, in the form of compact columns. The teliospores germinate *in situ*, each one producing a promycelium which gives rise to four sporida. A diagram of the life cycle is presented in text figure 1.[1]

In the United States and Canada the pine most frequently attacked is *Pinus strobus*, although *P. flexilis* James and *P. parviflora* Sieb. and Zucc. have been found infected. Practically every known species of Ribes is susceptible to infection to a greater or less degree, and therefore the discovery of an immune variety is much to be desired. The results of inoculations on *Ribes* spp. in America have been reported by Spaulding and Gravatt (*56*), and further work is being conducted with all the species and varieties of Ribes obtainable.

EXPERIMENTAL METHODS

The method used for rapid examination of specimens of pine suspected of being infected has been described in detail in a previous paper (7). This procedure in brief is as follows: Sections from fresh pine bark are cut on an ether freezing microtome, rinsed in water, stained in safranin and *Lichtgrün*, cleared in clove oil followed by xylol, and mounted in balsam. They are then examined, preferably with an oil-immersion lens, to determine the presence or absence of the characteristic mycelium and the striking haustoria of the rust, the latter being especially important from a diagnostic standpoint. This method yields transparent sections which for general morphological study have not been surpassed by following any of the more complicated methods given below. Very little shrinkage occurs in the mycelium, and as the method is usually employed before pycnia and æcia appear, the preservation of the hyphæ in as near their natural shape as possible is practically all that is necessary. Moreover, the host tissue shrinks so slightly that the distortion is entirely negligible.

Two killing agents were used in the preparation of material for paraffin or celloidin embedding: (1) formalin-alcohol, made by adding 6 cc. of full strength commercial formalin (U. S. P. VIII) to 94 cc. of 70 per cent

[1] The drawings for the text figures were made by the aid of a camera lucida and a special projection apparatus; for the drawings on the plates a camera lucida was used. The photographs and photomicrographs are all original. The author is indebted to Miss Minnie W. Taylor, Assistant in Forest Pathology, for the preparation of the drawing for text figure C of plate 56.

alcohol; and (2) Flemming's fluid, both weak and strong. The first was used for pine material only; the second for both pine and *Ribes* spp. Formalin-alcohol gave excellent result not only for gross mycelial characters but also in some cases for nuclear phenomena. The material sec-

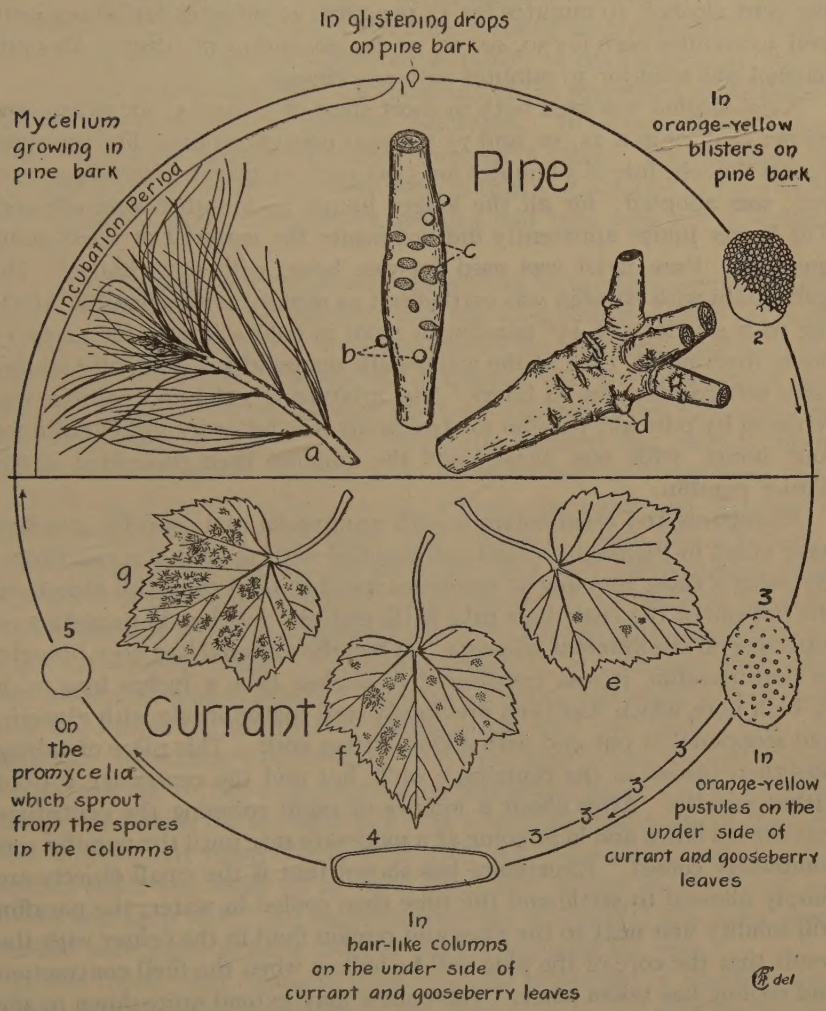

FIG. 1.—Diagram representing the life cycle of *Cronartium ribicola*. The spores are drawn to approximately the same scale and are numbered in the order of their appearance: 1, pycniospore; 2, æciospore; 3, urediniospore; 4, teliospore; 5, sporidium. The repetition of the figure 3 in the broken line between the urediniospore and the teliospore indicates that the urediniospores appear in successive generations in a single growing season. *a*, A young pine branch at the time infection usually takes place: *b*, pycnial drops; *c*, pycnial spots: *d*, æcia; *e*, the dots on the currant leaf represent uredinia; *f*, the dots represent uredinia and the dashes telial columns; *g*, this figure represents the condition of the infected leaf in the fall when the telial columns are the predominant spore forms.

tioned easily, either in paraffin or celloidin, and stained well with safranin and *Lichtgrün* or Haidenhain's iron-alum hematoxylin. Both concentrations of Flemming's fluid were very satisfactory. The material was cut into pieces not over 3 mm. square, fixed for 24 to 48 hours and then

thoroughly washed in running water. Certain generally accepted principles of killing and fixing were considerably modified by greatly shortening the time the material was left in the different reagents. The time the material remained in the grade alcohols was cut to 5 minutes for 10 per cent alcohol, 10 minutes for 15 per cent, 20 minutes for 20 per cent, and 30 minutes each for 30, 40, 50, 70, 80, 90, and 95 per cent. Absolute alcohol was used for 30 minutes with one change.

Xylol-alcohol was used both in short steps of 5, 10, 15, 20, 25, 50, and 75 per cent, or in a 25, 50, and 75 per cent concentration. For the short jumps the schedule of 5 minutes for 5 per cent, 10 minutes for 10 per cent, etc., was adopted; for all the longer jumps 30 minutes were allowed. The longer jumps apparently did not injure the material or its staining qualities. Pure xylol was used for one hour, with one change. The infiltration with paraffin was carried out as rapidly as was feasible, starting with shavings of 45° paraffin in xylol in cold solution, for 12 to 15 hours (overnight), then at the water-bath temperature, gradually adding more soft paraffin, for 24 hours. The mixture of xylol and paraffin was replaced by pure 45° paraffin for two hours, the latter by 55° paraffin for four hours, with one change and the material then embedded in the harder paraffin.

Æciospores and telial columns with promycelia and sporidia attached were killed in Flemming's fluid and handled with the aid of a centrifuge. By using a short spinning at moderate speed to send the small objects to the bottom of the centrifuge tube little material was lost in decanting or pipetting off the different reagents. When the material had been brought to pure paraffin, it was transferred to a glass tube 2 inches long with ¼-inch bore, which had been previously well coated inside with glycerin and stoppered at one end with a close-fitting cork. This piece of tubing was then placed in the centrifuge while hot and the centrifuge started rather rapidly. After about a minute of rapid spinning the centrifuge was slowed down and kept going at a moderate rate until the paraffin was completely cooled. Experience has shown that if the small objects are simply allowed to settle and the tube then cooled in water, the paraffin will solidify first next to the glass and remain fluid in the center with the result that the core of the plug will be hollow when the final contraction and cooling has taken place. The hollow may extend quite down to the cork, making it impossible to cut a clean, well-shaped block for sectioning. The use of the centrifuge completely overcomes this trouble and also crowds the minute objects closer to the bottom of the tube against the cork. When the paraffin has cooled, the cork is pulled out and the paraffin plug pushed out of the tube.

The paraffin method was used exclusively for embedding bark and leaf tissues. It was found that the bark tissue could be easily cut down to 5 μ without resorting to hydrofluoric-acid treatment. Wood was treated

with 50 per cent hydrofluoric acid and then embedded in celloidin,[1] or was simply placed in 95 per cent alcohol and glycerin, equal parts, for about 10 days to 2 weeks, and sectioned without embedding at all. Neither of these two methods gave better sections than could be obtained from fresh tissue with the aid of the ether freezing microtome and a sharp knife, except in the case of tangential sections. A thickness of 10 to 20 μ was found the most favorable for the study of the mycelium in the different elements of the cortex, phloem, and xylem. In sections thinner than 10 μ the mycelial strands were often torn out of place. For the study of the pycnia and æcia sections were used as thin as 3 μ, but 5 to 7.5 μ were generally employed. Sections of uredinia and telia were cut 3, 5, and 7 μ thick.

For mounting, Land's fixative,[2] potassium-bichromate + gum-arabic, was found superior to egg albumen and was used almost entirely for bark sections. It was also found more satisfactory for long sections of the leaf.

A rather long series of stains was employed, and a comparison of the different features of the mycelium and host cells made from slides colored with the different stains. The diagnostic method has already been mentioned. Alcoholic safranin and clove-oil + gentian-violet were particularly good for mycelium in the xylem. Safranin and Delafield's hematoxylin was also a favorable combination for such infected tissue. In the phloem and cortex the mycelium was well differentiated with Delafield's hematoxylin followed by erythrosin in 70 per cent alcohol. For cytological study Haidenhain's iron-alum hematoxylin in combination with aqueous Congo red, aqueous orange G, or *Lichtgrün* in 95 per cent alcohol proved excellent. Host and parasite cell walls stained well with the *Lichtgrün*, whether in combination with the hematoxylin or safranin. Flemming's triple stain[3] gave the best results for cytological study, with one or two exceptions to be mentioned later; and by using a strong violet stain the mycelial and host relations at the bases of the sori were brought out more clearly than with any other combination. The gentian-violet was made up in small quantities, enough for one week, as it does not keep well. All reagents from the safranin to the xylol were handled with pipettes from dropper bottles and used but once.

It was found that material once correctly embedded in paraffin, sectioned, and mounted, would stand the jump from 95 per cent alcohol to water, then a 5-minute immersion in full strength hydrogen peroxid for bleaching, without shrinkage or distortion. The sections were rinsed in

[1] See PLOWMAN, Amon B. THE CELLOIDIN METHOD WITH HARD TISSUES. *In* Bot. Gaz., v. 37, no. 6, p. 456–461. 1904.

[2] See CHAMBERLAIN, C. J. METHODS IN PLANT HISTOLOGY. ed. 3, 314 p., illus. Chicago, 1915.

[3] The stains were made up according to the following directions: SAFRANIN, Solution I: 2 gm. of safranin in 95 cc. of 95 per cent alcohol; filter. Solution II: 5 cc. of anilin oil in 95 cc. of distilled water. Shake the two solutions together and filter. GENTIAN-VIOLET, Solution I: 1 gm. of gentian-violet in 15 cc. 95 per cent alcohol. Solution II: 3 cc. of anilin oil in 80 cc. of distilled water. Mix the two solutions, shake, and filter. ORANGE G: 1 gm. of orange G. in 100 cc. of distilled water.

water after bleaching and kept in 70 per cent alcohol until they could be stained. They were stained in safranin for one to two minutes, rinsed in water, differentiated to the proper point with 50 per cent acid alcohol and washed with fresh 50 per cent alcohol. They were then stained for 15 to 60 seconds in gentian-violet. The violet was followed at once by the orange G with the slide held at an angle so that the orange G would run off rapidly and carry the violet with it. Then a few drops of absolute alcohol were mixed with the orange G which remained on the slide to hasten the removal of excess violet, and the mixture was quickly poured off. The mixing process and the pouring off of the mixture were carried out as rapidly as it was possible to manipulate the reagents and the slide. Then a few drops of fresh absolute alcohol were flowed quickly over the slide. The absolute alcohol was replaced by clove oil, the clove oil by xylol, and the mount then finished in balsam. The whole staining and finishing process consumed only a few minutes and the results were far superior to any obtained by following schedules calling for longer staining. The success of the method depends on having good material properly fixed, embedded, and sectioned, and on the use of fresh reagents. The saving in time and the excellence of the finished slides more than make up for the possible excess use of the more expensive alcohols and oils.

The method of using the iron-alum hematoxylin was also shortened by cutting the time in the mordant by from 10 to 30 minutes, and the time in the stain by from 5 to 30 minutes. The results were particularly good for the study of the centrosomes and dividing nuclei. It is obvious that the shorter methods outlined above have distinct advantages over the longer methods generally advocated, provided they yield satisfactory finished products. The short method [1] for the use of Flemming's triple stain is recommended to investigators who have had trouble with this more or less capricious combination.

MORPHOLOGY

INFECTION OF PINE AND EXTERIOR INDICATIONS OF PRESENCE OF PARASITE PREVIOUS TO SPORE FORMATION

The actual process of the entrance of the sporidial germ tube in infecting the pine has not been observed, and indeed the determination of the actual point of natural infection is an exceedingly difficult problem. Practically all infections first become evident by the etiolation or swelling, or both, of the bark at the node or at some point in the internode. The etiolation may be quite marked, as shown in Plate 48, A, representing a recent nodal infection. Internodal infections most often appear to originate at the base of a leaf fascicle. A striking case of this type of

[1] This short method, varied slightly according to the material to be stained, has been very successfully used at the University of Wisconsin and Columbia University, and the author makes no claim for its originality.

infection is shown in Plate 48, B, *a*. McCubbin (*29*) reports that out of 1,007 pine infections examined 925 originated at the bases of leaf fascicles, 14 originated in wounds, and 68 were indeterminate. These conclusions controvert the scarcely tenable theory of Marchal (*35*) that infection generally takes place through wounds. Observations on a large number of infections at Kittery Point, Maine, indicate that most infections can be classed as either nodal or internodal. However, it should be borne in mind that infection probably takes place very often in the bud while the nodal and internodal cells are scarcely differentiated. Therefore the point at which infection appears to originate after the tissues in the bud have elongated may be either nodal or internodal, depending entirely on the chance growth of the sporidial germ tube. In discussing *Peridermium pini* Pers., parasitic on *Pinus sylvestris* L., Hartig (*19*) states that infections with this rust also originate either at the node or the base of the leaf fascicle.

The swelling and etiolation of the bark noted above are the two most prominent indications in early stages after infection of the presence of the parasite. The swelling is confined to the bark alone, the wood actually becoming constricted in old infections, and is due to the fact that the mycelium of the parasite forces apart the phloem and cortex cells of the host. No evidence of any increased division has been observed in the bark cells, and there seems to be no stimulation toward gall formation, such as occurs in other cases—for example, on *Pinus virginiana* Mill. under the attack of *Peridermium cerebrum* Peck. In the case of the nodal infection in Plate 48, A, it will be noted that there is little or no swelling; but, as stated above, both swelling and etiolation may occur at the same time. The irregular edge of the etiolated area marks fairly definitely the advancing tips of the invading hyphæ, which generally extend a little beyond the line.

Other less common external indications of the parasite are bunched needles on seedlings or transplants and occasionally on older trees, and short adventitious branches which spring from the infected nodes. It frequently happens that certain secondary fungus parasites become established on the area already attacked by the rust, and suppress the latter so completely that spore production is partly or completely inhibited. Under these conditions the bark dies and shrinks more rapidly than under the attack of the rust alone, and the stem then appears to be constricted, more or less irregularly, at the infected part. All of these external indications are valuable aids to early recognition of *Cronartium ribicola*.

The period of incubation, from the time of infection to spore production, varies from one to several years, depending possibly on whether the infection takes place in early summer, and therefore under favorable conditions for growth before winter, or in early fall, when growth has unquestionably slowed down. The growth of the mycelium in the bark

in some cases seems to vary directly as the succulence of the host. It is at this time impossible to determine what part weather conditions play, although it seems evident that, once infection has taken place, external conditions cause little or no change in the growth of mycelium. The time of production of æcia in the spring varies directly with warm and favorable weather conditions.

MYCELIUM IN PINUS STROBUS

A brief summary of the main characteristics of the mycelium and the relation of the parasite to the cells of the cortex and phloem of the pine host has already been published (7); however, both subjects will bear review in greater detail. No data can be given at this time on the manner in which the mycelium enters the host tissue, because the youngest stages of infection have not been observed. The course of the hyphæ is always intercellular (Pl. 53). Conditions found at the edge of the infected area indicate that the fungus makes its advance first in the most recent phloem parenchyma and in the rays. This holds true for infections of several years' standing, as well as for those which, to judge from the size of the diseased area, could not have been more than a year old. The hyphæ are relatively large, 3 to 5.5 μ in diameter, and are divided by cross walls into cells which vary considerably in length (Pl. 53). Each cell has a single conspicuous nucleus. In transverse and radial sections the hyphæ can be traced along the rays past the cambium into the xylem (Pl. 53). Here they are confined to the region of the ray cells, with the exceptions noted below. Because of difficulties in cutting transverse sections, which are at right angles not only to the "grain" of the phloem but also to the general course of the hyphæ, such sections are not favorable for study, except in comparison with other sections cut tangentially or radially. In tangential longitudinal sections of the cortex and phloem the mycelium is frequently found in strands, which are especially prominent in the outer phloem region. The strands may surround groups of the phloem parenchyma. In some cases the older sieve cells appear to be filled with hyphæ; but careful observation shows that they have been split apart and flattened out by the hyphæ, which have forced their way into the enlarged intercellular space, and thus practically occupied the same amount of space that was formerly occupied by the living sieve cells. In the rays the hyphæ frequently fill the intercellular spaces adjacent to the horizontal walls of the ray cells, and haustoria from these hyphæ penetrate the ray cells. Resin cells are also penetrated by haustoria. In the xylem the same general conditions are met with in respect to the ray cells that are found in the phloem. Short haustoria arising from the hyphæ in the angles between these ray cells bore through the thin walls of the adjacent tracheids and enter the lumens

of the tracheids [1] for a short distance (Pl. 49, C). Such haustoria are naturally limited in development.

Radial longitudinal sections are more easily cut from bark or wood than transverse or tangential longitudinal sections because radial cuts are splitting cuts. Moreover, in radial sections the vertical widths of the rays are exposed, and therefore all the infected cells of the rays, with the haustoria they contain, as well as the adjacent infected phloem and xylem cells, are more readily examined (Pl. 53). The hyphæ lying along one phloem ray frequently are united with similar hyphæ lying along adjacent rays by connecting strands. The latter may work their way between phloem parenchyma or sieve tubes. In the xylem the ray hyphæ may be connected in the same way by hyphæ, which pass from one ray to the other in the intercellular spaces between the tracheids. Often the edge of the ray is bordered by a hypha lying in the space between the outer ends of the marginal cells of the ray and the tracheids (Pl. 53). In the resin-duct parenchyma and the cells lining the duct all the cells are usually penetrated by haustoria (Pl. 53). This applies to vertical ducts in the wood and bark and to horizontal ducts in fusion with rays.

The general features of the mycelium such as their more or less uniform diameter, with occasional bulges where there is a little extra room in an intercellular space, their relatively large size, uninucleate cells, and constant relations to the host cells of the different tissues, are characteristic enough to make the mycelium alone a sufficient basis for the recognition of *Cronartium ribicola* before any spores are produced. The haustoria often the most striking objects in the infected cells, are the most important elements of the mycelium from a diagnostic standpoint. The haustoria apparently have the power to pierce the cell wall at any point (Pl. 53). Young haustoria are usually straight (Pl. 58, B), constricted at the point of passage through the wall, and irregularly bulging inside the cell (Pl. 53; 58, C, D). Their outline soon becomes more or less irregular. In the phloem parenchyma they do not reach the development found in the ray cells, where they coil on a wide spiral or curve at rather sharp angles. The curve and the spiral are probably different expressions of the same process—that is, the adjustment between the growing haustorium and the cytoplasm of the host cell. As Sappin-Trouffy (*50*, *51*) has pointed out, the haustoria of the rusts appear to seek out the nucleus of the host cell, and sometimes even entwine it. No such extended development has been observed in the case of the haustoria under discussion, but it frequently happens that the host nucleus is dented (Pl. 53) by the tip of the haustorium. Olive (*42*) shows that the haustoria of *Botryorhiza hippocrateae* Whetzel and Olive form botryose masses which may almost completely fill the host cell. In this fungus the haustoria

[1] In a previous article (*7*) the statement was made that haustoria do not enter the wood cells. Improved technic has shown this statement to be an error.

are multinucleate; in *C. ribicola* they are apparently always uninucleate (Pl. 53). In the latter fungus no case of more than one cell in a haustorium has been observed by the writer, although as many as three tips are not uncommonly seen. It is difficult to determine whether the haustorium is always cut off from the hypha from which it is originated and this point will have to be left undecided.

When properly stained with any of the combinations given on page 623, a sheath can be made out at the base of each haustorium (Pl. 58, C, D), enveloping it for a distance of several microns, as if the haustorium were set in a cuplike holder. The sheath really extends all over the haustorium, but is generally very thin in the middle region. At the tip it is as thick or thicker than at the base (Pl. 58, E). In its staining reaction the sheath resembles the wall of the host cell. There is at least a possibility that it is formed by the host cytoplasm in response to the irritation or stimulus caused by the presence of the haustorium. Olive (*42*) states that the plasma membrane of the host protoplasm is pushed in by the haustorium of *Botryorhiza hippocrateae* as it invades the host cell. Apparently the cytoplasm shrinks away from the advancing haustorium in some cases, leaving an appreciable space. This phenomenon has not been observed in *Cronartium ribicola*, but it is certain that the plasma membrane of the host cell is not broken nor pierced by the haustorium; It must be pushed in as the tip of the haustorium grows. The greatest points of irritation produced by the haustorium in the host cell would be the point in the cell wall through which the haustorium entered and the point of contact of the advancing haustorial tip with the host cytoplasm. Consequently it might be assumed that the greatest results from the irritation would be observed at these two points—namely, at the base of the haustorium and at its tip. At these two points the sheath is thicker than at others. Possibly the narrow space which must occur between the haustorium and the host cytoplasm membrane is the dumping ground for precipitation products resulting from the irritation. These products might constitute the beginning of the sheath which would gradually increase in thickness as the age of the haustorium increased.

The sheath certainly does not come into being at the time the haustorium penetrates the wall, as Smith (*53*) has reported for the haustorial sheaths of the Erysipheae. It has not been found in connection with young haustoria in any case and seems to be an accompaniment of maturity or old age. The hole in the host cell wall, through which the young haustorium passes, is comparatively small (Pl. 58, C, D) and at first the wall is no thicker at this point than at any other. Whatever thickening takes place at the point of penetration occurs after the haustorium has entered the cell.

The young haustorium is full of cytoplasm, with a typical round nucleus (Pl. 58, B). As the wall of the haustorium, which is at first

quite thin, thickens and the sheath develops, the cytoplasm becomes vacuolate (Pl. 58, C, D, E), and the nucleus loses its normal structure, condensing into a shrunken deeply staining mass. In such a state the haustorium can not function efficiently as an absorbent organ. At any rate the host cell and its nucleus do not show much evidence that the parasite seriously interferes with the normal cell growth. Even when the cells are completely separated by mycelial stands, the host nuclei may remain apparently normal in shape and structure.

PYCNIA

On any given area of infection the pycnia precede the æcia, by at least one growing season. Succeeding generations of pycnia and æcia follow a more or less definite schedule. The plan of the advance of the fungus is illustrated in Plate 54, A. Immediately surrounding the point of infection the bark may show pycnia and æcia at the same time (Pl. 48, B), but in such cases the pycnia have either passed maturity or the æcia have developed abnormally early. After the infection has spread for some distance, provided that the tree is not less than several inches in diameter, the pycnial area is normally always in advance of the æcial area. The sequence of the etiolated bark, pycnial area, and æcial area is evident in Plate 54, A.

Very young pycnia are difficult to find. They seem to develop rapidly when once started, for none have been examined which were mature in the center and still young at the periphery, a condition which is commonly met in æcia and uredinia. The hyphæ which contribute to formation of the pycnium force their way between the cells of the outer cortex in a direction at right angles to the outer surface of the bark (Pl. 49, B, *a*). They are aggregated between the outermost cortex cells and the periderm, forming a layer of pseudoparenchyma two to four cells thick (Pl. 49, B, *b*). From this layer arises a series of short branching trunks (Pl. 58, A, *c*). Each of the ultimate branches from these trunks is a long sporophore, on the tip of which a number of pycniospores are formed, one after another (Pl. 49, A, B; 58, A, *a*, *b*). Roughly speaking, the pseudoparenchyma makes up one-fourth, the short branching trunks one-fourth, and the sporophores one-half of the vertical width of the sorus (Pl. 49, B, 58, A). Pycniospores are produced in large numbers. Mixed with a thin sweet gelatinous fluid, they collect between the sporophore tips and the periderm layer (Pl. 49, A) forcing the latter up into the form of a shallow blister. Finally a small break in the periderm layer allows the spores to escape, together with the sweet fluid, in the form of a honey-colored drop called the pycnial drop (Pl. 48). The pycniospores are typically pyriform (Pl. 58, A, *b*), measuring when mature approximately 2.5 by 3.5 μ. Their mode of formation appears to agree closely with that of the pycniospores in other rusts. At the rounded tip of the sporophore a bud is formed which swells until it reaches the size of a mature spore

(Pl. 58, A). After receiving its nucleus the spore is abstricted. No evidence of a collar like that figured by Blackman (*2*) was seen, unless the odd constriction of the cytoplasm shown in Plate 58, A, *c*, can be considered as a collar. This phenomenon is quite common. Occasionally long hyphal filaments grow some distance out beyond the tips of the sporophores. The structure of the spores is typical of pycniospores in general. They appear to be completely nonfunctional. No attempt was made to germinate them.

The dark areas on the bark which indicate the location of the pycnia are designated pycnial spots (Pl. 54, A, *b;* 48, B). They are honey-yellow to brown-yellow at first, but they gradually assume a color like that of clotted blood as the pycniospores mature and ooze out, and may finally become almost black. Later, when the covering of cork cells sloughs off or is eaten off by insects, the drying cells of the host tissue beneath turn a typical light pink color. After reaching maturity the pycnium is cut out from the host tissue by the formation of a cork cambium and the deposition of a cork layer at a depth of four to six cells below the bottom of the pseudoparenchyma base of the sorus (Pl. 50, C, *b*). It is the exposure of this layer which reveals the typical color of newly formed cork cells. The pycnial spots, whether young or old, are valuable diagnostic characters, because they often make possible the detection of infected trees before æcia are produced—that is, in time to destroy such trees before æciospores can spread the disease.

ÆCIA

Æcia appear in April, May, and June. Very often the whole area on which æcia can normally develop in a given season is covered with closely crowded sori pushing their way through widening cracks in the bark. By the middle of May the peridia are usually broken and the spores escape in orange-yellow pollen-like showers. Spore production continues for some time after the æcia open. Young æcia are easily obtainable at the edge of the æcial area where their presence is indicated by a thin yellow line just beneath the outermost layers of the bark cells. The hyphæ contributing to the formation of the æcium are aggregated into a mycelium, which is clearly made up of elements running tangentially among the host cells at a depth of 6 to 10 cells below the periderm layer. By their continued growth these hyphæ force the host cells apart, so that the latter become isolated and embedded in a matrix of tangled mycelium (Pl. 50, B). This separation may extend to a depth of 15 to 20 cells in the cortex. About 6 to 8 cells below the periderm layer a mass of pseudoparenchyma is formed by the packing together of the hyphæ. In the pseudoparenchyma a layer of fertile cells becomes discernible by their denser protoplasmic content (Pl. 50, A, *b;* 54, B, *fc*).

The fertile cells cut off rows of sterile cells (Pl. 54, B, *sc*), 6 to 12 cells long, which may increase in size at first, but which later degenerate to make

room for the developing æciospore chains. These sterile cells correspond to the buffer cells of Olive (*41*) and Fromme (*14*, *15*). The cells of the fertile layer, which are somewhat larger than those of the vegetative mycelium, may fuse in pairs, the fusion beginning in the center of the sorus and proceeding centrifugally toward the periphery, as is normally the case in the æcium and its analogs. As a result of the fusion and union of the cell contents of the contributing cells a large cell is formed, the basal cell (Pl. 58, I, J), which will give rise acropetally, by repeated division, to the æciospore chain. The fusion seems to be complete as far as the cells are concerned, and evidently takes place rapidly, as there is only occasional evidence even in very young æcia of remaining parts of the cell walls which originally separated them (Pl. 58, I). The two cells are not always at the same stage of development, as one of the cells is often shorter or at a lower level than the other (Pl. 58, J). However, there is little ground for considering the two cells as different in kind. The essential process is the cell fusion and consequent association of the nuclei to form the dikaryon [1].

Fusion of more than two cells also occurs, trinucleate and tetranuclaete basal cells being quite common. Æciospore chains arising from such basal cells may contain the same number of nuclei as the basal cells, just as in æcia of other genera. Trinucleate basal cells are very numerous in young sori and at the edge of older ones, but the number of trinucleate æciospore chains is considerably less than one would be led to expect from the number of the trinucleate basal cells. There is a possibility that the extra nucleus frequently degenerates, evidence of this being occasionally seen. This point will be discussed briefly later on. Multiple cell fusions of a more complex character are also common (Pl. 58, K, L) recalling Olive's (*41*) and Fromme's (*14*) observations. The nuclei and cytoplasm of a number of adjacent cells, not only those in the upper row of the fertile layer but also others considerably below that level, become associated in one large irregular cell (Pl. 58, L). What happens to these large cells is not clear. The probability that the multiple fusion cells may give rise to a number of spore chains is reservedly suggested here.

FORMATION OF PERIDIAL CELLS AND ÆCIOSPORES

The basal cell divides into an upper part, the æciospore initial cell, and a lower part, potentially equivalent to the primary basal cell (Pl. 58, M). Each of the first few cells cut off from the basal cells normally divides into two cells of unequal size. The larger cells thus formed adhere more or less completely into a layer three to five cells thick which constitutes

[1] The term "dikaryon" is to be preferred to that of "synkaryon," the more common term, because of the earlier use of the latter name to designate the fusion nucleus resulting from the union of the male and female gamete nuclei by zoologists. Pavillard's (*43*) objection to synkaryon should be sustained, and Maire's (*33*, *34*) suggestion that dikaryon be substituted for the older word has the virtue of correcting an error and supplying a distinctive term for the unique condition found in the rust sporophyte and the Basidiomycetes in general.

the peridium (Pl. 56, B); the smaller ones go to pieces. At the periphery of the sorus several adjacent basal cells cut off units which never become functional æciospores but which always form peridium. The small cells between these peridial cells indicate that the potential æciospore initial, although destined to become a peridial cell, regularly divides to form two cells homologous to an æciospore and an intercalary cell (Pl. 56, A). Thus, the multiple-layered peridium of *Cronartium ribicola* is formed in the same general manner as reported by Fromme (*15*) for the peridia of other deep-seated æcia. When first formed, the individual cells are subspherical to elliptical, and smooth-walled. As the sorus matures, their walls thicken and their outline changes according to their position in the peridium. All tend to become more or less polyhedral. The cells at the top of the sorus are usually more rounded than those at the sides, since the latter are elongated by the pull exerted on the sides as the developing spore chains force the central part of the peridium out. Therefore, the size of the cells varies widely (18 to 40 by 12 to 42 μ). The mature wall is 3 to 9 μ thick. The walls of the outermost cells are smooth or slightly granular, while the inner cell walls are studded with short tubercles which sometimes appear to mesh with those of the adjacent cells, perhaps contributing thereby to the strength of the peridium as a whole (Pl. 56, B). The cell contents of the peridial cells slowly degenerates until they become empty shells.

After the cells which form the peridium are abstricted from the basal cells, the æciospore initials are cut off. Each æciospore initial cell undergoes division into a larger upper cell, the æciospore, and a smaller lower cell, the intercalary cell (Pl. 58, W, M, *b*, X, Y, *c*, *d*). By the repeated divisions of the basal cell a row of alternating spores and intercalary cells is formed which constitutes the æciospore chain (Pl. 50, B; Pl. 58, Y). After the division of the æciospore initial cell, both resulting cells, the æciospore and intercalary cell, grow rapidly. The æciospore reaches its normal broadly elliptical shape when about three or four spores distant from the basal cell. The intercalary cells elongate, eventually becoming mere thin connecting elements between the æciospores in the chain, and finally disintegrate entirely. The spore wall thickens greatly when the spore has attained its full size. A thin space in the wall, suggesting the germ pore of other rust spores, is evident at the point of attachment of the æciospore and the intercalary cell below it (Pl. 58, Y, Z). This thinner place in the wall may persist even in the completely matured spore. It is comparable to similar phenomena in other rusts and does not normally function as a germ pore. The mature spore measures 18 to 21 by 20 to 26 μ. The æciospore wall seems to be made up of two parts, an endospore overlain by a somewhat thicker exospore. The latter is distinctly characteristic of æciospores of *Peridermium* spp. Part of it is cracked up into tubercles or warts, which makes it decidedly verrucose, and the rest of it is smooth (Pl. 58, Z). The smooth

area is more or less indefinite in extent at the basal end of the spore. Verrucose and smooth areas grade one into the other along the irregular line which separates them. The smooth area is fissured near its edge into blocks which become smaller and smaller until they approach the size of the tubercles of the verrucose area (Pl. 58, Z).

No completely satisfactory explanation of the manner in which this type of spore sculpturing arises has come to the writer's attention. The following theory is reservedly offered. The two walls of the spore are present when the spore is quite young, and both continue to grow and thicken up to a certain point or until the spore has nearly reached its full size. The outer wall hardens more rapidly than the inner one and in consequence becomes fissured irregularly as the still elastic inner wall continues to expand under the pressure of the growing spore content. The longer growth in size continues the more complete will be the fracturing process and the larger the verrucose area in proportion to the smooth area. Experimentally, the smooth area can be converted into verrucose area by soaking the spores in water. After several hours, if germination does not take place in the meantime, the spore absorbs water enough to cause it to increase appreciably in size. The tubercles of the verrucose area become free from their attachment to the inner wall and float around in the water. The smooth area, under the expansion pressure exerted as the inner wall swells, cracks and fissures until it becomes irregularly verrucose, approaching the conditions found on the normal verrucose area of the mature spore. The process of fissuring can be watched quite easily. The experiment at least suggests the manner in which a type of spore sculpture so oddly irregular could arise.

The spore wall on the smooth area has been heretofore considered to be thicker in section than the verrucose area. Examination of complete sections of the spores shows that this is not always the case. In fact, it is only in occasional instances that it holds true. As a rule, there is no appreciable difference in the thickness from the inner edge of the wall to the outer tip of any given tubercle and the thickness from the inner edge of the wall to the outer edge of the smooth area. When dealing with whole spores, refraction phenomena increase the difficulties in measuring the true thickness of a curving wall of the type presented in the smooth area of the æciospore of *Cronartium ribicola* and may account in part for the misinterpretation of the actual condition.

GERMINATION OF ÆCIOSPORES

Germination of the æciospores may take place rapidly under favorable conditions, but as Maire (*32*) aptly puts it "*la germination des ecidiospores*. * * * *est parfois très capricieuse.*" The work of Spaulding and his assistants has shown that spores which would not germinate at all in hanging-drop cultures, on a water film, or on moist filter paper, either at room temperature or in the ice box, or at room temperature after cooling

in the ice box, were perfectly capable of producing infection on species of Ribes. These results confirm those of Klebahn (*25*), who found that æciospores which would not germinate in water did germinate very rapidly on leaves of *Ribes* spp. and only less rapidly but still abundantly on a gelatinous decoction of leaves of *Ribes* spp. These experiments suggest the probability that some direct chemotactic stimulus is exerted by the leaves of *Ribes* spp. on the æciospores. Klebahn has pointed out that there is considerable difference between the faculty for germination, as determined by artificial cultures, and the faculty for infection. Spaulding and his assistants have further determined that æciospores frequently germinate—even then only a relatively low percentage of fresh spores do so—more readily after cooling in the ice box than at room temperature, and that sometimes they have to remain in the ice box to secure germination. Too much water is often as inimical to germination as too little. A single spore may produce one to several germ tubes (Pl. 59, A), which attain considerable growth in artificial cultures. Where the germ tube passes through the heavy exospore it is constricted as shown in Plate 59, B. The tubes branch freely. The protoplasm is densest at the advancing tips of the hyphæ.

INFECTION OF RIBES SPP. AND MYCELIUM IN THE LEAF

Whether the germ tubes have the power to pierce the upper epidermis of the leaf of *Ribes* spp. or must always come to rest in a favorable position on the lower epidermis in order to cause infection is not definitely known. All the evidence gathered from the examination of artificially inoculated leaves points to the conclusion that infection occurs normally as a result of the germination of the æciospore on the lower surface of the leaf and the subsequent passage of the germ tube through a stoma. No evidence either of any break in the upper or lower epidermal cells, or of the remnants of any hypha passing through them, has been discovered. Furthermore, the mycelium is always abundant in the air chambers adjacent to the stomata, even in very young infections, and occasional remnants of spores and hyphæ near and in the stomata point to the stomata as the avenue of infection.

The first indication of infection in the leaf of species of Ribes is often indicated by the paling of the infected areas. Sections of such areas show that the mycelium has spread in the intercellular spaces and air chambers of the mesophyll. Haustoria (Pl. 59, D) enter all types of the leaf cells, with the possible exception of the xylem elements of the bundles, although they are comparatively rare in the epidermal cells. The cells of the mycelium and the haustoria are binucleate (Pl. 59, C, D). Generally there is a much smaller relative amount of mycelium in the leaf tissue than among the same number of host cells of the pine. The loose structure of the mesophyll allows plenty of room for the hyphæ to grow without severe crowding of the host cells. In fact, the hyphæ are aggregated only at the time of production of uredinia or telia.

URÉDINIA

The development of the uredinium is illustrated in Plate 51, A, B, and 55, A–C, which form a series from the very beginning of the formation of the sorus to its maturity. When the uredinium starts to form, the fungus cells may be found aggregated in groups in some large air space, generally near a stoma. Certain of the cells in each group become oriented with their long axis more or less at right angles to the epidermis, against which they are closely appressed (Pl. 55, A). These cells are functionally equivalent to the basal cells of the mature sorus, in that from them arise the cells which go to make up the peridium, and the first urediniospores. The first division of any one of the vertically elongated cells results in the formation of a cell which adheres to its neighboring homologous cells to make the peridium (Pl. 55, B, *a*). In the second division the first urediniospore initial cell, or its equivalent, is cut off. This divides immediately to form the first urediniospore and stalk cell or their equivalents. The position of the cells referred to, as they appear in a young uredinium, are illustrated in Plate 55, B, in which *a* is the peridial cell, *b* the young urediniospore or its homolog, *c* the stalk cell or its homolog, and *d* the basal cell. It will be noted that the arrangement of the cells suggests that the sorus is made up of a compact aggregation of vertical rows of cells. This arrangement is temporary for the middle region of the sorus, but permanent for the circumference of the sorus. Plate 55, B, which represents a median section from a young uredinium, can be duplicated by taking sections through the edge of any mature sorus. This fact should be kept clearly in mind in any discussion of the structure of the uredinium.

The first urediniospores mature in the middle of the sorus (Pl. 51, B, *b*). Their formation and method of growth corresponds closely with the production of normal stalked urediniospores in other rusts. To repeat the process suggested above: The basal cell undergoes division (Pl. 55, C, *c;* 59, E–I). The upper cell is the urediniospore initial (Pl. 59, J, L); the lower cell is potentially the equivalent of the original basal cell. The urediniospore initial now divides (Pl. 59, K, L, M) to form a larger upper cell, the urediniospore, and a smaller lower cell, the stalk cell (Pl. 51, B, *b, c;* 55, C, *d*). While this process is going on, the layer of cells constituting the peridium gradually separates from the underlying urediniospores along the line between the cells marked "*a*" and "*b*" in Plate 55, A. The figures in Plate 51, A and B, represent steps in the process leading up to conditions shown in Plate 55, C. As the urediniospores grow, the peridium (Pl. 51, A, *a;* B, *a*) is forced up into a dome. The individual peridial cells lose the regular shape and outline shown in Plate 59, B, *a*, and become irregularly compressed or obliquely flattened. The growing urediniospores develop pressure against the epidermal cells, which flattens them out and finally causes them to be torn apart (Cf. Pl. 51, A, B, with Pl. 55, C). The break in the epidermis frequently

comes at a stoma, sometimes extending from one stoma to another; but the break in the peridium, when it finally occurs, is confined more or less definitely to the top of the dome. Usually the peridial cells around the break are irregularly thickened (Pl. 55, C). The orange-yellow urediniospores work their way out through the opening, sticking more or less closely to each other to form a spore crown on the top of the peridium. The basal cells continue to cut off urediniospore initials by a process similar to that involved in the formation of the primary initials, which has been described above. The secondary urediniospore initial may be formed alongside the base of the stalk of the first-formed urediniospore before the latter has reached maturity (Pl. 55, C, *c;* 59, N). The secondary initial then divides to form a urediniospore and stalk cell, in the same way as the primary initials. Meanwhile the stalk cell of the primary urediniospore elongates, withers, and goes to pieces. While no more than two spores have been found in connection with a single basal cell, each basal cell must frequently give rise to several spores, to judge from the number produced in a single sorus. The size of the mature spore (Pl. 59, P) is 10 to 20 by 19 to 45 μ.

The spore-bearing basal cells are confined to the middle part of the sorus. In figure C of Plate 55 it will be noted that on either side of the group of spores there is a group of parenchyma-like cells (*g*) made up of units which are arranged in more or less vertical rows. The cells of any individual row of four cells may be homologized with the cells lettered *a*, *b*, *c*, and *d* in figure B of Plate 55. This parenchyma-like tissue forms an encircling bank of cells which completely surrounds the mature uredinium. In this tissue the row arrangement of the cells shown in figure B persists, although the cells themselves lose their contents and become practically dead by the time the basal cells in the middle of the sorus are actively forming urediniospores.

The uredinium seems to be limited more definitely than either the pycnium or æcium in its ability to extend in a centrifugal direction. Its extent is predetermined, much more exactly than in the case of the two other sori, by the amount of massed mycelium from which it arises. Both pycnia and æcia originate in tissue which is comparatively full of mycelium before they start to develop. In the leaf of species of Ribes there is rarely any massing of the hyphæ to form the packed mycelium so common in the pine host, and the leaf cells are only occasionally distorted except in the immediate vicinity of the uredinia and telia.

GERMINATION OF THE UREDINIOSPORES

Urediniospores exhibit the same irregularity in germinating in artificial cultures as do the æciospores. Even when they are dusted over the surface of young, fresh, moistened leaves and placed in a damp chamber they may or may not germinate in large numbers. On a water film they absorb water and swell considerably. The cytoplasm becomes vacuo-

late. The germ tube passes through the exospore without the aid of a germ pore, assuming at first the shape of a more or less swollen vesicle (Pl. 59, Q). This lengthens rapidly into the young germ tube (Pl. 59, R), into which pass the contents of the spore. The endochrome and protoplasm are densest at the tips of the tube and in its branches. Duff (*12*) has shown that change of temperature stimulates the urediniospores to germinate and that light, especially ultra-violet light, may completely inhibit germination. This action of light may have a direct bearing on the apparent failure of the spores to cause infection at long distances from their source. All the evidence at hand points to the conclusion that the germ tube causes infection by passing through the stomata on the lower surface of the leaves. However, the tube may extend some distance over the leaf surface before actually entering a stoma, in such cases passing directly over stomata in its path. Urediniospores may retain their viability for some weeks. The uredinium is the repeating sorus in the life cycle of *Cronartium ribicola* (fig. 1), and the spores in succeeding generations infect leaves of *Ribes* spp. until late summer.

TELIA

Telial columns arise either from old uredinia or as entirely new and separate entities. They appear later than the uredinia and are more common in late summer. In the fall they are usually the predominant spore generation present. In the greenhouse they are produced throughout the year. On a given infected area the columns may occupy the central part where the uredinia were first produced, surrounded by a narrow peripheral region bearing the most recently formed uredinia. The development of the telial column is the same whether it is from an old uredinium or in a new sorus and, as the latter is probably the most common occurrence, it will be described. The massing of the hyphæ, formation of the peridium, and the parenchyma-like cells which surround the spore-bearing part of the sorus, and the orientation of the basal cells proceed exactly as in the uredinium. It is impossible to tell very young uredinia and telia apart. The binucleate basal cells undergo division (Pl. 59, S, T, U, V, W) in the same manner as in the case of the uredinium, but the cells cut off do not divide as in the other sori described. Instead they lengthen out and become teliospores. Each basal cell cuts off a vertical row of spores (Pl. 52, A, B), the central cells of the sorus producing spores slightly ahead of the cells at the periphery, as in the æcium and uredinium. As the spore columns lengthen, the peridium is pushed up into a dome (Pl. 52, A), which later ruptures (Pl. 52, B) and goes to pieces. The spores lengthen and soon reach full size, at about which time they become provided with a substantial wall slightly thickened at the upper end (Pl. 57, A, B, C). The first spores cut off—those at the tip of the column—are more or less polyhedral (Pl. 52, B; 57, A); the other spores are typically broad spindle-shaped (Pl. 52, D;

57, B). They usually touch, but do not crowd one another. Adjacent to the points where one spore abuts on its neighbors there may be considerable space. Cross sections of the columns (Pl. 52, E) show that the spores are only very slightly angular as a result of mutual pressure. Although they vary widely, normal mature teliospores average approximately 16 by 42 μ, with a wall about 2 μ thick. They are uninucleate, as a result of the fusion of the two nuclei which are normally present in the young teliospores. The mature telial column is an aggregation of a number of vertical rows of mature teliospores (Pl. 52, C). The length and diameter of the columns vary greatly; they may attain a length of 2 mm. and an average width of approximately 100 μ. They are usually curved or semispiraled, a result apparently of unequal development of the spores of some of the rows. They vary from almost spherical to irregular ovoid or elliptical in cross sections. Occasionally abortive, nondeveloped spores are scattered throughout the length of the column (Pl. 52, C, D). Other abnormalities will be discussed briefly later.

GERMINATION OF TELIOSPORES AND PRODUCTION OF SPORIDIA

All of the spores in the telial column may germinate *in situ* (Pl. 56, C). The exospore pushes out at some point in the form of a rounded papilla, which ruptures and allows the extrusion of a stout germ tube—the young basidium or promycelium (Pl. 57, D). This reaches its full size in a few hours and then becomes divided into five cells (Pl. 57, R) From each of the four upper cells arises a stout sterigma, on the tip of which the sporidium swells to its full size (Pl. 57, S–V). When abstricted, the sporidium is almost exactly spherical, measuring approximately 8 to 10 μ in diameter. At one point on the thin wall is a tiny papilla-like swelling, which marks the point of attachment to the sterigma (Pl. 57, AA, EE, GG).

The germ tubes from the spores in the middle of the column work their way out through the intersporal spaces and then develop in the manner described above. If the teliospores germinate under water, the germ tube lengthens out into a narrowly spiraled or twisted hypha; in other words, promycelia do not develop unless they have access to the air. The color of the germinating column, a very pale pink, gives a distinctly characteristic appearance to the fungus at this stage.

GERMINATION OF SPORIDIA

The sporidia germinate in artificial culture by sprouting relatively stout germ tubes (Pl. 57, X, Y, Z, BB) which probably continue growth in the normal fashion under favorable environment. In many cases, however, the short germ tube swells at its tip on reaching a length of several microns and the swelling becomes a secondary sporidium, apparently exactly similar to the primary one (Pl. 57, CC, DD). Sappin-Trouffy (51) has figured the same phenomenon for the sporidia of *Cronartium flaccidum*

Alb. and Schwein. on *Peonia officinalis*. It is apparently a common occurrence. Just how the sporidia reach the pine is not known, but it may be assumed that they are usually air borne. Infection of the pine host follows their germination under favorable circumstances on young pine shoots.

CYTOLOGY

PREVIOUS INVESTIGATION

Our knowledge of the cytology of the genus Cronartium is very fragmentary; in fact, no reference has been found to the nuclear phenomena accompanying the formation of any bark-inhabiting æcium of this group. Poirault and Raciborski (*44*) figure silhouettes of the nuclear division, as they interpreted it in the formation of the æciospores of the form known to them as *Peridermium pini acicolum*.[1] Sappin-Trouffy (*51*) gives a fair diagram of this acicolous type of æcium and the nuclear division at its base. His diagram of the telial column of *Cronartium flaccidum* indicates the phenomena accompanying the production of teliospores, though the figures are too minute to be more than suggestions of the actual conditions. He regards the processes as closely similar to the nuclear phenomena in other rusts, in which he is quite correct. Aside from the work of these authors, nothing beyond incidental mention of the cytology of the genus Cronartium has come to the writer's attention.

It is hardly necessary to give an extended resumé of the work of previous investigators on the cytology of the rusts, on account of the excellent reviews which have been published by Blackman (*2*), Christman (*4, 5, 6*), Fromme (*14, 15*), Maire (*32*), Guilliermond (*16*), and Ramsbottom (*46*). Moreau (*36, 37, 39*) revives the older view that there are but two chromosomes in the rust nucleus, which hardly seems tenable, and establishes the presence of centrosomes in the resting nuclei (*36*). This subject will receive further consideration later. Fromme's (*15*) recent paper on the morphology and cytology of the æcium clears up the matter of the formation of the deeper seated sori and establishes the similarity of spore formation in the cæoma and æcium. The life history of a complete rust is divided into two stages: the gametophyte, with uninucleate cells, and the sporophyte, with binucleate cells. The gametophyte begins with the reduction division, or its equivalent, in the promycelium and continues up to the fusion of the gamete cells at the base of the æcium, which initiates the dikaryon. The sporophyte begins with the inception of the dikaryon and continues up to the reduction division, or its equivalent, in the promycelium. The association of the nuclei in the basal cells of the æcium is regarded as the equivalent of a true fertilization, and the fusion of the two nuclei in the young teliospore as the completion of the process necessary for a mixing of the chromatin elements previous to reduction (*31*). The nuclear divisions are true mitotic divisions, accom-

[1] Vuillemin (*62*) also reports some of the phenomena accompanying æciospore formation in *Peridermium pini*.

panied by centrosomes or their equivalent, true spindles, and chromosomes. The character of the latter is still unsettled. In the dikaryon both nuclei divide simultaneously in conjugate division.

The chief differences of opinion have been in regard to the nature of the two cells which fuse to form the basal cells and in regard to the sterile cells. Blackman (*2*) held that the two basal cells were unequal, either in size or time of development, and that one, the larger, received the nucleus of the other, the smaller, by a process of nuclear migration through a comparatively small pore. After Christman's paper (*4*) announcing the fusion of two equal cells by the complete absorption of their appressed walls, Blackman and Fraser (*3*) investigated a number of forms and stated that the dikaryon might arise (a) through the process of nuclear migration from one cell to another, as Blackman first reported, or (b) by a similar nuclear migration from one vegetative cell to another below the fertile layer, or (c) by the process described by Christman. Most of the evidence since brought to view has supported Christman's theory. Olive (*41*) has pointed out that the manner of fusion is not of great importance, inasmuch as the conjugating cells are not definitely organized as male and female organs; for the essential result of the fusion is the establishment of a dikaryon by the association of two nuclei and their accompanying cytoplasm, regardless of their individual origin.

The sterile cells cut off from the fertile cells before fusion have been regarded as homologous to the trichogyne of the Florideae (*2*); as buffer cells which protect the developing basal cells below (*15*, *41*); or as degenerate female gametes, which once were fertilized by the now nonfunctional pycniospores. According to this last theory, advanced by Moreau (*40*), the cells as a group constitute a preæcium (*preecide*) and the individuals preæciospores (*preecidiospores*). Fromme (*15*) has shown that the number of sterile cells varies considerably in different species and suggests that they are formed in response to the general conditions of environment under which the particular sorus happens to be developed.

In the following presentation of the cytological details in *Cronartium ribicola* the fusion nucleus in the mature teliospore will be taken as the starting point, inasmuch as it is the first stage in the nuclear history of the gametophyte generation. The nuclear processes accompanying the production of the different spore forms can then be described in logical cytological sequence and at the same time in chronological order.

NUCLEAR PHENOMENA IN FORMATION OF PROMYCELIUM AND PRODUCTION OF SPORIDIA

With the germination of the teliospores the morphological history of the gametophyte may be said to begin. Coincident with this germination, changes occur in the fusion nucleus. As the promycelium reaches

its full size the fusion nucleus, formed by the union of the two nuclei in the young teliospore, migrates from the teliospore into the promycelium and starts to divide (Pl. 57, E). The membrane becomes irregular and disappears (Pl. 57, F) at the same time that the chromatin granules increase in size. These granules become longer and more deeply-staining units, which form a tangled mass in the position occupied by the fusion nucleus when it moved out into the promycelium (Pl. 57, G). A definite spindle, with centrosomes at the poles, then develops. At the middle of the spindle the chromatin tangle becomes arranged in an equivalent of the equatorial plate stage. In the next stages the chromatin is in the form of separate units (Pl. 57, H, I), which can be clearly seen under favorable conditions and proper differentiation. Plate 57, I, shows 16 such units which apparently are equivalent to distinct chromosomes. They separate into two groups, and the elements of the groups migrate along the spindle to the corresponding poles (Pl. 57, J, K). Radiations from the region of the centrosomes are present, but not easily stained during metaphase (Pl. 57, I), and quite long and prominent throughout anaphase (Pl. 57, K) and at telophase (Pl. 57, L, M). They seem to be due to cytoplasmic condensation rather than of the nature of true astral rays. The two chromatin groups in late anaphase are condensed into irregularly lobed masses (Pl. 57, L, M) at the ends of a more or less curved strand of suspension fibers, recalling Blackman's figure 31, Plate 21, (*2*). The suspension fibers disappear, the daughter nuclei reorganize around the chromatin groups as centers, and a wall divides the young promycelium into two cells (Pl. 57, N). A second division follows immediately (Pl. 57, O, P, Q). The two pairs of granddaughter nuclei thus formed are then separated by cross walls (Pl. 57, R), and the 4-celled promycelium completed. As a rule, the empty basal portion of the promycelium is also cut off by a wall, so that the promycelium really has five cells, four active and one, the stalk, practically dead. Each nucleus is relatively small, with a definite nucleolus and a minute centrosome. The contents take the form of a fine granular network.

From each of the four active cells of the promycelium a stout sterigma is protruded (Pl. 57, S), on which a single spherical sporidium is formed (Pl. 57, V). The nucleus of the cell migrates through the sterigma (Pl. 57, U), taking on an irregular shape during the process, and then rounds up into its normal form (Pl. 57, AA). The division of this nucleus to form a binucleate sporidium (Pl. 57, GG) is quite common, and the karyokinetic figures are particularly striking. Early and late anaphase stages are represented in figures EE and FF, Plate 57. The nuclear behavior in the formation of secondary sporidia was not followed. In germinating sporidia the nucleus probably migrates into the germ tube and there divides, although the figures of this process were indefinite and unsatisfactory. Figure BB of Plate 57 illustrates a common condition which indicates that the nucleus is preparing to divide, at the same

time moving toward the germ tube. At present no data can be given on the young mycelium from the uninucleate or binucleate sporidia, and this comparison must remain impractical until the process of sporidial germination and infection of the pine can be more closely followed.

NUCLEAR PHENOMENA IN VEGETATIVE MYCELIUM IN PINUS STROBUS

The uninucleate mycelium produced in the bark of *Pinus strobus* following infection by the sporidia has already been described. The nuclei are completely organized, with a definite nucleolus, chromatin network, and membrane (Pl. 57, HH, II). The chromatin appears to be more or less definitely centered on one point on the nuclear membrane where the centrosome is located. This condition of the arrangement of the chromatin elements of the nucleus will be referred to as polarization, and will be discussed in connection with its appearance in other stages in the cytological cycle. Few cases of vegetative division have been observed in the vegetative cells, but the stages of the process which have been seen indicate that there is a typical rust spindle and that the division is comparable to the vegetative divisions described by Olive (*41*).

NUCLEAR PHENOMENA IN PYCNIUM

The nuclei in the pseudoparenchyma layer from which the short branches bearing the pycnial sporophores arise are quite similar to those of the vegetative mycelium in shape, size, and organization, but they are colored more intensely with hematoxylin and the violet of Flemming's triple stain. The nucleus of the pycnial sporophore is relatively large for the size of the sporophore (Pl. 58, A). In its resting state the chromatin is scattered throughout the whole nucleus in minute granules, which are rather difficult to stain clearly. Each nucleus has a definite centrosome indicated by the local condensation of the chromatin (Pl. 58, A, *a*). The pycniospore (Pl. 58, A, *b*), which swells to its full size on the tip of the sporophore, receives one of the daughter nuclei resulting from the division of the sporophore nucleus (Pl. 58, A, *d*). The exact details of the process were not definitely followed, on account of the small size of the pycniospores and the narrow passage from sporophore to spore. The mature subpyriform pycniospore has the typical structure of corresponding forms in other genera of the rusts. A relatively large nucleus is surrounded by a small amount of cytoplasm (Pl. 58, A, *b*).

NUCLEAR PHENOMENA IN ÆCIUM

In the layer of fertile cells and in the mycelium below this layer the nuclei are somewhat larger (Pl. 58, H) and more readily stainable than those of the deeper vegetative mycelium (Pl. 57, HH). All the nuclei in this region of the layer and below it exhibit the phenomena of polarization, although the fact may not be evident unless the profile view can be seen. The same condition persists in all other nuclei in or near the vari-

ous sori. Above the fertile layer a series of cells, the sterile cells (Pl. 58, G), arranged in more or less definite vertical rows, are cut off (Cf. Pl. 54, B, *sc*). Fromme (*14*) finds that the sterile cells in *Melampsora lini* (DC.) Tul. arise from the division of the first sterile cells; Moreau (*40*) states that in *Phragmidium subcorticium* they are formed by the division of the cells in the fertile layers. In *Cronartium ribicola* they seem to arise from the cells of the fertile layer (Pl. 58, F), but there is some evidence that the sterile cells themselves also divide occasionally. Each row contains from 6 to 12 cells, whose contents degenerate as the æciospores mature. The pressure of the peridium and the lengthening æciospore chains flatten the whole tissue of sterile cells against the overlying cortex cells.

CELL FUSION TO FORM BASAL CELLS

Fusion normally occurs between two adjacent cells of the fertile layer, which may or may not be at the same level (Pl. 58, I, J). The walls between the fusing cells appear to dissolve, leaving one large cell where there had been two. The basal cell thus formed is the initial cell of the dikaryon. The nuclei are comparatively large (cf. fig. G and I, Pl. 58). They exhibit the polarization phenomena referred to above, but their content is less easily stainable than in the vegetative nuclei below the fertile layer (cf. fig. H and I, Pl. 58), for at times the space between the nucleolus and the membrane seems almost empty (Pl. 58, Y, *a*). When the nuclei are located in dense cytoplasm toward the upper end of the basal cell, the lower end may be occupied by one or more large vacuoles (Pl. 58, Q). When fusion of more than two cells occurs, the nuclei usually are more irregularly placed (Pl. 58, K, L). The nuclei in these compound basal cells and in the multiple fusion cells are similar to those in the normal binucleate cell.

THE CONJUGATE DIVISION IN THE BASAL CELLS

The division of one of the nuclei of the basal cell will be described, it being understood that the companion nucleus undergoes the same changes at the same time; that is, that the division process is a conjugate division typical of the rusts. On the nuclear membrane is a body which stains deeply with hematoxylin—the centrosome (Pl. 58, I). From it in most cases there run strands of chromatin which are more dense than the other chromatin elements of the nucleus. The nucleus increases in size and the membrane bulges, except at the points where the chromatin strands touch it (Pl. 58, Y, *a*). At this stage the membrane becomes very thin and then disappears, fading first on the side away from the centrosome (Pl. 58, M, *a*). Meanwhile the chromatin elements group around or at one side of the nucleolus. Fine suspensors appear to connect the centrosome and the condensing chromatin mass. In the left nucleus in Pl. 58, M, *a*, the centrosome appears to have divided into two. Coincident with

the disappearance of the nuclear membrane the nucleolus is cast out (Pl. 58, N), after which it migrates off to some distance from the chromatin mass. A true spindle is then formed at the two ends of which are located the centrosomes (Pl. 58, O, P). The original centrosome was not seen to divide, but the conformation of the spindle in some early stages suggests the division of the centrosome to form two, which move apart and become oriented as the opposite poles of the spindle. The chromatin now appears in deeply stained sections as a convoluted dark mass at the middle of the spindle (Pl. 58, O). With better differentiation this mass resolves itself into a jumble of more or less rounded units, the chromosomes (Pl. 58, P). This stage corresponds to the equatorial plate stage of the metaphase. The chromosomes are now seen to separate and move toward the poles (Pl. 58, Q–S); they seem to flow along the outer surface of the spindle rather than to be drawn definitely apart by attraction fibers. Unless very carefully differentiated, the chromatin at this stage may appear to be a single knotted thread reaching from pole to pole. The chromosomes, however, do not fuse into one mass, but, as can be seen in favorable preparations, remain as separate units (Pl. 58, R). As they approach the poles the chromosomes apparently arrange themselves into two groups (Pl. 58, T) for each pole. The division of the centrosomes before the daughter nuclei are reorganized may explain the locus of the two groups, as suggested by Olive (*41*). At this late stage of anaphase the whole figure may resemble the silhouette dumbbell figures by earlier investigators of the subject. The two groups at each pole now become condensed to two deeply staining masses (Pl. 58, U). Fibrous connections between the daughter groups may still remain visible. The cast-out nucleolus has persisted in the cytoplasm up to this time, slowly becoming less dense and often decreasing in size, while the process described above has been taking place, but as the last spindle fibers disappear and the daughter nuclei begin to become reorganized it fades away, completely absorbed by the cytoplasm. The companion nucleus of the dikaryon, having divided simultaneously with the one described, has by this time given rise to two other daughter nuclei. The four reorganizing nuclei now move apart, two and two, the sister nuclei separating and moving in opposite directions. A wall now forms (Pl. 58, V, *a*), separating the upper pair from the lower, forming the æciospore initial and, below it, a new basal cell (Pl. 58, M), potentially equivalent to the primary basal cell.

STEPS IN FORMATION OF ÆCIOSPORE CHAIN

Division of the dikaryon in the æciospore initial follows the same process as that in the basal cell (Pl. 58, W, M, *b*, X). The wall which forms between the two pairs of nuclei divides the original initial cell into two parts, an upper cell which is the æciospore and a lower, which is the intercalary cell (Pl. 58, Y, *d*, *c*). Repeated division of the basal cell and

æciospore initials gives rise to a row of alternating æciospores and intercalary cells (Pl. 58, Y), as has been stated in the description of æciospore formation elsewhere in this paper. The nuclei of the æciospores soon become completely reorganized (Pl. 59, AA) and typical of the dikaryon in all its stages, but the nuclei of the intercalary cells slowly degenerate and disappear. A centrosome, the center for the polarization phenomena previously mentioned, can be differentiated by proper staining on the membrane of each nucleus.

NUCLEAR PHENOMENA IN UREDINIUM

The mycelium in leaves of species of Ribes has already been described. The nuclei at this stage of the dikaryon are only slightly less in diameter than that of the hyphæ, and they are therefore somewhat separated—not side by side as in the elements of the æciospore chain (Pl. 59, C). They do approach each other in the larger cells at the base of the uredinium and undergo conjugate division by a process apparently identical (Pl. 59, E–I) to that described for the basal cell and æcispore initial of the æcium. The same holds true for the division in the urediniospore initial (Pl. 59, J–M, N, *b*). The wall formed between the two pairs of daughter nuclei divides the initial into an upper, larger cell, the young urediniospore, and a lower, smaller cell, the young stalk cell, which rapidly elongates. The nuclei in the spore become organized similarly to those of the æciospore (Pl. 58, AA), while the stalk nuclei slowly degenerate (Pl. 59, N). The second and subsequent conjugate divisions in the basal cell (Pl. 59, O) are similar to the primary division, but the nuclei often are not so definitely placed side by side, an irregularity probably due to the tendency of the cytoplasm flowing into the second urediniospore initial to pull one of the nuclei along with it. As a rule, both in the basal cell of the æcial chain and in the basal cell of the uredinium at the time of the primary division, the nuclei, though they may commence to divide when located at different levels in the cell, become arranged side by side at metaphase. In the second division of the urediniospore basal cell this orientation may not take place, so that at telephase one of the daughter nuclei may be well up in the new urediniospore initial and its companion just passing into it; but on reorganization after the initial is cut off from the basal cell, the two nuclei take a position side by side in the typical manner. Their division and the subsequent cutting off of the stalk cell by a wall give rise to a second urediniospore. This process may be repeated several times, for the basal cell seems to retain its powers of division until the sorus dries up.

NUCLEAR PHENOMENA IN TELIUM

The telia are borne on the same mycelium that gives rise to uredinia. By an exactly similar process of conjugate division (Pl. 59, S–W) a cell is cut off from the basal cell of one of the telial unit columns; but this

cell does not divide again, being in itself the young teliospore (Pl. 59, X). The two nuclei appear to become fully reorganized (Pl. 59, Y). They then fuse immediately to produce the single large nucleus of the mature teliospore, during which process the nuclear membranes are absorbed at the points of contact, so that the nuclear contents are free to mix (Pl. 59, Z). The fusing contents round up and become surrounded by the reorganizing membrane. In Plate 59, Z, the two centrosomes and the two nucleoli are still visible. The process of the fusion of the latter was not observed. The fusion nucleus increases in size in a remarkable manner immediately after the union of the two contributing nuclei; and the enlargement may continue until the volume of the fusion nucleus is as much as four times the combined volumes of the two contributing nuclei. Its diameter at the time of maximum enlargement nearly equals the diameter of the teliospore. The chromatin at this stage stains very heavily and appears to be condensed into a heavy irregular spireme-like structure, in which the individual strands are frequently knotted (Pl. 59, AA). Holden and Harper (*21*), in discussing the fusion nucleus in the teliospore of *Coleosporium sonchi-arvensis* Lev. (*C. solidaginis* (Schw.) Thum.?), present evidence that the heavy skein breaks up into long pieces, which later split longitudinally into finer threadlike units. In *Cronartium ribicola* there certainly occurs a marked reduction in the thickness of the chromatin threads, but no process which could be interpreted as actual splitting was clearly seen. As the chromatin becomes more finely drawn it contracts into a more compact tangle, the nucleus shrinking meanwhile (Pl. 59, BB), and finally splits into granules (Pl. 59, CC). The nucleus at this stage is nearly spherical; the nucleolus is rather small, and is generally located near the membrane. The centrosome could not be differentiated in the fusion nucleus with any stain. So far as could be determined by careful examination, it does not reappear until the primary division in the promycelium and in the resting nuclei of the promycelium.

DISCUSSION OF CYTOLOGICAL PHENOMENA

The nuclear phenomena accompanying cell fusion and spore production in *Cronartium ribicola* clearly confirm the views generally held in regard to rust cytology. Fusion of the gamete cells in pairs in the bark-inhabitating æcium parallels closely similar phenomena in other types of æcia and analogous sori, while multicellular fusion is perhaps much more common than in other forms investigated. Fromme (*14*) has called attention to triple-cell fusions and the fact that cells below the fertile layer often contribute to the multinucleate fusion cells of *Melampsora lini*. The behavior of the fusion cells in *C. ribicola* shows that cells below those of the fertile layer are potential gametes. It is quite certain that the multiple fusions observed are regular occurrences in æcia of all sizes and shapes, whether on roots or stems. Dittschlag (*11*) and Hoffman

(*20*) both figure two æciospore chains rising from a large basal cell in *Puccinia falcariæ* and *Endophyllum sempervivi*, respectively, and there is some evidence that aeciospore chains often arise in like manner in *C. ribicola*. It is hardly possible, however, to compare the multiple fusion cells to the central placental cells reported by Richards (*49*). Inasmuch as the elements of the æciospore chains are generally binucleate, the number of polynucleate spores being relatively small, it must be concluded that either the extra nuclei so common in the basal cells degenerate or the complex basal cell gives off more than one binucleate spore chain.

The constant similarity in the process of congugate division in the basal cells of the æcium, uredinium, and telium suggests a definite stability of the nuclei of the dikaryon throughout its existence. The presence of the centrosome in the resting and dividing nuclei adds confirmation to the reports of this structure in rust nuclei as given by other writers. While it is impossible to make a definite statement from actual observation of the process, it seems perfectly evident that the centrosomes at each pole may divide, thus forming two loci for the chromatin groups approaching the poles—cf. Olive's figures 5, *a*, and 10, Plate 22 (*41*). The theory that these two groups of chromatin represent two simple chromosomes, the interpretation of the phenomenon apparently accepted by Mme. Moreau, does not appear tenable, unless it is assumed that these chromosomes are compound and that they break up into their components at metaphase and reunite at telophase. The observations of Holden and Harper (*21*), Blackman (*2*), Christman (*4*), and Olive (*41*) and the evidence presented in this paper establish the presence of more than two chromosomes or chromosome equivalents, possibly eight being the haploid number. Moreau's (*37*) figures indicate that her preparations were apparently not sufficiently differentiated to show all the details in the chromatin masses.

The polarization of the nuclei, so evident in nearly all of the resting stages, recalls the condition illustrated in Olive's figure 8, Plate 22 (*41*). The arrangement of the chromatin with a point on the membrane as a locus for the convergence of the strands is not due to fixation; for it is a constant phenomenon in nuclei killed and fixed under different conditions and with different reagents. The centrosome is apparently the center of attraction. Harper (*18*) has conclusively demonstrated similar phenomena in the Erysipheae. He has also called attention to the fact that the nuclei of these ascomycetes exhibit a definite regularity and stability with reference to the arrangement of the chromatin strands and the polarization throughout the cytological changes which he observed. He believes the chromosomes retain their individuality to a marked degree in all the nuclear processes. The observations on the nuclear structure in *Cronartium ribicola* herein presented suggests that

perhaps the conditions in the rust nucleus approach more nearly than has been supposed the conditions in the Ascomycetes and other fungi. The process of nuclear fusion in the young teliospore is comparable to the same process in other rusts. It seems reasonable to conclude that this fusion is the completion of the process, initiated at the time of cell fusion to form the dikaryon, which takes the place of normal fertilization, and that the actual fusing is a necessary preliminary to the mixing of the chromatin elements and the subsequent reduction division, as suggested by Maire (*31*) and others. Certainly the changes which take place in the nucleus after fusion suggest a complicated mingling and readjustment of the chromatin which would seem to justify such a view. It is not necessary to regard the fusion as a pseudo fertilization (*10*). There appears to be little reason for doubting that the first division in the promycelium is heterotypical (*38*), for it is unique and decidedly different from the second division which immediately follows. Arnaud (*1*) has compared the mitotic figures in *Capnodium meridionale* with those in *Coleosporium senecionis*. Wager's figure 84, Plate 19 (*63*), of the telophase in the dividing fusion nucleus in the sporangium of *Polyphagus euglenae* is very like similar stages in the primary division in the promycelium of *Cronartium ribicola*. It may be noted that the association of the nuclei in *P. euglenae* and their subsequent fusion in the sporangium are phenomena comparable to the nuclear conditions in the rust dikaryon and fusion in the teliospore.

ABNORMALITIES

Before coming to the general discussion and summary it is necessary to mention briefly certain abnormalities commonly met in the different types of sori. Æcia sometimes occur with reversed polarity in part of the sorus, where the spores are produced on æcial chains which grow toward the center of the tree, usually into a resin duct. This change in the direction of growth is probably to be explained by the fact that the developing chains followed the line of least resistance, in this case into the adjacent resin duct, instead of against the overlying host cells. Æcia also often develop on the roots, under several inches of leaves and loam. Their structure appears to be normal, but their environment is hardly advantageous. Double pycnial layers (Pl. 50, C, *a*, a_1) are not uncommon. In a short note, Posey, Gravatt, and Colley (*45*) have reported the finding of uredinia on the stems of *Ribes hirtellum* Michx. In the cortex of infected stems of this species internal uredinia (Pl. 51, C) with normal and reversed polarity were formed in abundance. Internal telia, produced in the pith and cortex of the petioles of *Ribes* sp.,[1] have been described in a previous paper (*8*). In such abnormal

[1] *Ribes roezli* was the name given in the original article, but the species determination was probably incorrect. As the species has not fruited, accurate determination has been impossible.

sori, typical column development is sometimes completely inhibited by the pressure of the surrounding host tissue. All internal sori are to be regarded as teratological phenomena of no special morphological significance. Uredinia and telia on the petiole are common; occasionally they develop on the upper surfaces of infected leaves.

GENERAL DISCUSSION

The writer's observations on the mycelium of *Cronartium ribicola* in the bark of *Pinus strobus* confirm the conclusions of Klebahn (*25*) in regard to the course of the hyphæ of the parasite and add considerable new data on the inter relations of the host and parasite cells. Hartig's paper (*19*) on *Peridermium pini* and Wolff's article (*64*) on the same fungus, which appeared shortly after Hartig's and in some places is almost a direct copy of Hartig, are the only papers which have come to the writer's attention which describe and illustrate the morphology and parasitic relations of a bark-inhabiting rust and its pine host. Hartig believes that the swelling of the bark of the host is due to the fact that the cortex and phloem cells are forced apart by the abundant intercellular mycelium of the parasite. This has been shown to be the case with the hypertrophy produced by *C. ribicola* in the bark of *P. strobus*. Observations on the bark of *P. parviflora* Sieb. and Zucc. infected with the same fungus show that the swelling is produced in the same way as it is in *P. strobus*. The same statement holds true for the swelling caused by *C. comptoniae* Arth. in the bark of *P. sylvestris* L., and *P. ponderosa* Laws. Neither *C. ribicola* nor *C. comptoniae* are gall-forming rusts. Their mycelium is confined almost entirely to the region of the cortex and phloem cells of their pine hosts, although the hyphæ do enter the wood along the rays, as Tubeuf (*58*) has reported for *Peridermium pini*, and occasionally work their way in between tracheids. These hyphæ have been traced in the case of *C. ribicola* to a depth of three annual rings, counting in from the cambium. In all probability it will be found that the mycelium may be found in the ray cells of the annual ring laid down at about the time infection took place, although it may not remain active after the ray cells die. While the presence of the hyphæ in a given annual ring may not mean that the pine was infected during the year this annual ring was laid down, because there must be some growth along the ray cells toward the center of the tree, it will establish roughly the date of infection. The similarity which evidently exists between the morphology and method of parasitism of *C. ribicola* and *C. comptoniae* suggests that a close agreement will probably be found among all non-gall-forming caulicolous Peridermia.

The actual injury to the pine host cells from the irritation caused by the invading mycelium is apparently very slight. The cells pierced by

haustoria continue to remain alive and apparently active in spite of haustoria. As has been pointed out above, these haustoria become invested with a sheath which may render them very ineffective absorbing organs, in which case they might become simple mechanical irritants in the cell content, no more detrimental to cell activity than crystals. The denting of the host nucleus by the tips of the haustoria does not seem to injure the nucleus except to alter its shape. There is no evidence of increased cell division reported by Reynolds (*48*) for many plants as a direct result of a parasite's action, or of nuclear migrations like those figured by Schürhoff (*52*). Storage starch is usually present in excess, but this phenomenon has been shown by Halsted (*17*) to be a general condition in and around areas infected with fungus parasites. This starch is not completely used up by the fungus, for many grains remain in the old dead cells after the cells are completely dried out. The cells just beneath the pycnial layer and in the region of the young æcium (Pl. 52, B) contain normal grains as well as decomposition products. Excess starch production is probably due to a lack of balance in the physiological processes in the host cells, and the fungus may, of course, contribute to the unbalancing; but other environmental factors which are little understood result in excess starch production in trees which appear to be perfectly normal in other respects.

The wood laid down in the annual rings under infected bark is much less than in healthy trees, but the tracheids are apparently normal in everything except number. The presence of the hyphæ in the tracheids has no appreciable influence on their form; neither are the characters of the ray cells perceptibly changed.

Cronartium ribicola may be the primary cause of the death of a young tree. However, the swelling of the bark is not in itself a serious hindrance to conduction in the phloem. The actual severe injury occurs when the æcia form and burst through the outer bark; for the æcial cracks thus formed allow the inner bark cells to dry out and die. This results in the breaking of resin canals and the consequent exudation of resin in large quantities. The girdling of the tree is due to these two causes working together—namely, the cracking and drying of the bark, and the impregnation of the whole cortex and phloem in the cracked area with resin. Complete stoppage of the conducting elements of the phloem results. Seedlings and young trees may succumb to the attack of the fungus almost as soon as the first æcia appear; but with older trees death is sometimes delayed for a number of years. It depends, of course, on how complete a girdle has been effected. The part played by secondary fungi acting in conjunction with *C. ribicola* to make a girdle complete is very important. It will be recognized at once that the cracking of the bark at the time the æcia are formed is a source of danger to the trees not only by exposing the inner bark cells to the air, but also by providing an avenue of entrance for secondary

parasitic fungi, or saprophytic fungi, and insects, which hasten the decomposition of the weakened susceptible tissue. These secondary fungi may also gain entrance through the pycnial spots. Rathay (*47*) has shown that the pycnia of many rusts are visited by insects, apparently attracted by the sweet drops. Both pycnia and æcia in *C. ribicola* may be eaten out by various insects, and unquestionably these insects bear on their bodies or legs viable spores of fungi capable of growing in the bark tissue. In many instances these fungi are so rapid in their growth that they overrun the infected area and completely suppress the rust, so that it never forms æcia. In such cases, the bark shrinks and the infected stem is actually constricted, and the girdle thus formed is often more quickly effective than in the cases where rust works alone.

In leaves of *Ribes* spp. the mycelium sometimes causes the death of isolated infected spots; but in other cases the hyphæ penetrate to all parts of the tissue without causing death of the cells, and without producing hypertrophy. The large spaces among the mesophyll cells and the fact that the hyphæ rarely form solid mycelial masses in leaves of *Ribes* spp. probably help to explain the lack of hypertrophy and destructive effect. When defoliation occurs during the course of severe epidemics of the rust, there is, of course, a consequent poor crop of berries. The variation in the effect of the parasite on the different species of Ribes is a subject which must have separate treatment and therefore can not be considered fully at this time.

It will probably be found on further investigation that a close agreement exists among caulicolous Peridermia with respect to the structure of the pycnium. The external appearance of the pycnium, or of the thin layer of tissue overlying it, will, however, be found to vary according to the outer bark texture of the host. In the case of *Pinus parviflora* the exterior appearance of the pycnium is almost identical with the pycnium of *P. strobus*. The value of the pycnial spots as diagnostic characters has been briefly outlined in an earlier short note (*9*), and referred to above in connection with the discussion of the formation of the pycnium and pycniospores. Hartig (*19*, *Taf. 4*, *fig. 7*, *b*) was one of the first observers to call attention to the pycnial spots on the bark of *P. strobus*, although he at that time thought the fungus on this pine was identical with *Peridermium pini*. Wolff (*64*) copied the same figure in his paper on the latter fungus. Kirchner and Boltshauser (*22*) in Plate 15 of their atlas show what are evidently three pycnial spots, but they do not definitely refer to them in their description of the figures. The ability to recognize the pycnial spots of *Cronartium ribicola* is almost absolutely essential in the field study of control methods.

The description of the formation of the æcium given above emphasizes the remarkable agreement which exists in the fundamental processes involved in the production of æciospores in the rusts. The figures of Hartig (*19*), Wolff (*64*), and Sappin-Trouffy (*51*, *fig. 65*) on the æcium of

Peridermium pini are not complete nor detailed enough to enable one to compare directly the structure of the acicolous and caulicolous æcia with the structure of the æcium of *Cronartium ribicola*. It is evident, however, that the leaf and stem types are quite similar. Study of the expansive æcia of *C. occidentale* Hedgc., Bethel, and Hunt may reveal some interesting morphological variations.

The details given for the formation of the multilayered peridium are apparently the first published record of the origin of this structure. Emphasis should perhaps also be placed on the constant and normal occurrence of multinucleate cells at the base of the æcium, a phenomenon which has been discussed elsewhere, by suggesting that in deep-seated æcia of caulicolous Peridermia the æciospore chains may be found to arise more often from these placenta-like cells than from basal cells arising as a result of the fusion of only two cells in the fertile layer. Although recent investigators—for example, Kurssanow (*27*)—have confirmed the results of Christman (*4*) and other writers with regard to the origin of the basal cell as a result of the fusion of two adjacent fertile cells, it yet remains a question whether this method is constant or whether certain variations in the formation of the basal cells are to be expected in deep-seated æcia.

Ludwig and Rees (*28*) in a recent article report some details of the structure of the uredinium of *Pucciniastrum agrimoniae* (Schw.) Tranz. Their figure would serve very well for a figure of the young uredinium of *Cronartium ribicola* (cf. Pl. 55, B). In the description of the uredinium of the latter it has been shown that the peridium is formed by the coalescence of cells which are cut off from certain cells that are analagous to the basal cells of the sorus. These cells also cut off urediniospore initials which then divide into urediniospores and stalk cells. In the young sorus these four cells—that is, the basal cell, the stalk cell, the urediniospore, and the peridial cell—form what looks like a chain of cells. As the spores and their stalks mature, the row arrangement is lost in the middle of the sorus, but persists at the circumference. This fact places the conclusion of Ludwig and Rees that the urediniospores of *P. agrimoniæ* are borne in chains, under suspicion. Personal investigation by the writer into the structure of the uredinium in this species of Pucciniastrum shows that the method of formation of the urediniospores in *P. agrimoniæ* and in *C. ribicola* is practically identical, and that therefore the spores in the uredinium of the former are not borne in chains but on stalks. In the case of *P. agrimoniae* the stalks are sometimes quite short and the basal cells from which they arise are much less conspicuous than they are in *C. ribicola*, but these differences are not important as far as the method of spore formation is concerned. The encircling bank of parenchyma-like cells surrounding the uredinium in *C. ribicola* is not found in *P. agrimoniae*. It is difficult to interpret Magnus's (*30*) figures of the uredinia of the Pucciniastrum group as to the exact morphology of the cells from

which the urediniospores arise, but his description of the manner in which the urediniospores in Pucciniastrum are borne is correct. Fischer's (*13*) figure of the uredinial peridium of *C. ascelpiadeum* (Willd.) Fr. evidently represents the same type of peridium as is present in the uredinium of *C. ribicola*. The peridial cells surrounding the break in the top of the peridium in the latter fungus are thickened irregularly, but they do not often appear as conspicuous as the large cells figured by Fischer (*13*) in the case of the uredinial peridium in species of Pucciniastrum or by Ludwig and Rees (*28*) for *P. agrimoniae*.

In the discussion of the telium it has been shown that it originates in a way which makes it impossible to tell the young telium from the young uredinium. Under these circumstances it is most natural that a peridium should be found over the telium, as in the case of the young uredinium. The same holds true for the parenchyma-like bank of cells which encircle the base of the column. Both the peridium and the bank of encircling cells come into being before the sorus becomes differentiated into either uredinium or telium. With these facts clearly in view it may be reasonably safe to predict that a telial peridium, a structure which has apparently not been reported previously, will be found to be present in other species of the genus Cronartium. The manner of the germination of the teliospores, previously discussed, adds to our knowledge of the morphology and behavior of the spores in the telial column, at the same time confirming the observations of Tulasne (*61*) and Sappin-Trouffy (*51*) on other species.

This paper is offered as a contribution to our knowledge of the parasitism, morphology, and cytology of the rusts, and especially of the genus Cronartium. It is hoped that the data presented may prove valuable in stimulating further research on the interrelations of rust parasites and their hosts, which will, of course, involve more accurate study of the anatomy of the hosts and the modifications in the normal structure of the host tissues under the action of the parasites. The need for a comparative paper on the haustoria of the rusts scarcely requires emphasis. It will be interesting to compare the results of investigations on the cytology of other deep seated caulicolous æcia with those herein presented, especially with reference to the process of the formation of basal cells, the phenomena of polarization of the nuclei, and the centrosomes and chromosomes.

SUMMARY

(1) In the foregoing paper hitherto unpublished data on the morphology and cytology of *Cronartium ribicola* Fischer and the interrelations of the parasite and its hosts, *Pinus strobus* and *Ribes* spp., are presented and fully illustrated.

(2) The mycelium is more abundant in the *Pinus strobus* than in species of Ribes. In the former the hypæ force the cortex and phloem

cells apart and thus cause the swelling of the infected bark; in the latter there is rarely any marked aggregation of the hyphæ, except in the case of petiolar infection.

(3) Haustoria may penetrate practically every cell in the infected area in *Pinus strobus*. These haustoria are characteristic for *Cronartium ribicola* and their presence in the bark cells of the pine definitely determines the identity of the parasite. A sheath develops around each haustorium as it reaches maturity or old age. This sheath is apparently unlike any other sheath so far described in connection with the haustoria of the rusts.

(4) Haustoria are proportionately much less frequent in *Ribes* spp. than in *Pinus strobus* and much smaller in size. They are not enveloped in sheaths as in the latter.

(5) The morphology of the different sori is shown to be similar to the morphology in other full-cycle rusts.

(6) The structure of the spreading pycnial layer characteristic of caulicolous Peridermia is considered in detail.

(7) The development of the deep-seated æcium and the formation of its multilayered peridium is described.

(8) The formation of urediniospores is shown to follow the general plan in other uredinia where the spores are borne on stalks. The development of the uredinial peridium and the bank of parenchyma-like cells which surround the uredinium are described and figured for the first time.

(9) The telial column is shown to arise either from an old uredinium or as a separate entity and to be indistinguishable from a young uredinium in its very early stages. It is surrounded at the base with a bank of parenchyma-like cells and is provided with a peridium similar to the peridium of the uredinium. All of the spores of the telial column may germinate *in situ*. The production of sporidia is described in detail.

(10) The destructive effect on the pine host as a result of the attack of *Cronartium ribicola* varies. In young trees death may result quickly. In older trees it is of the nature of a primary injury, which prepares the way for the drying out of the infected bark and the entrance of secondary fungi and insects which complete the destruction initiated by the parasite.

(11) The effect on Ribes varies with the species attacked. It may result in early defoliation and a consequent poor crop, but in general it is not serious on this host.

(12) The cytological phenomena presented herein establish the similarity of the nuclear processes in the genus Cronartium and other full-cycle rusts.

(13) The resting nuclei in all cases, with the exception of the mature fusion nucleus in the teliospore, have a deeply staining spot on the

membrane called the centrosome. This body is apparently the center for the more or less definite convergence and concentration of the chromatin strands referred to as polarization.

(14) The presence of large, irregular, multinucleate cells at the base of the æcium is a regular phenomenon and not to be regarded as an abnormal condition. Probably more than one æciospore chain may arise from such cells.

(15) There is a remarkable constancy in the process of conjugate division and stability in the nuclear structure throughout the dikaryon. In the dividing nuclei the centrosomes are visible as deeply staining dots at the poles of the spindle. The number of chromosomes is certainly more than two. Possibly eight is the haploid number.

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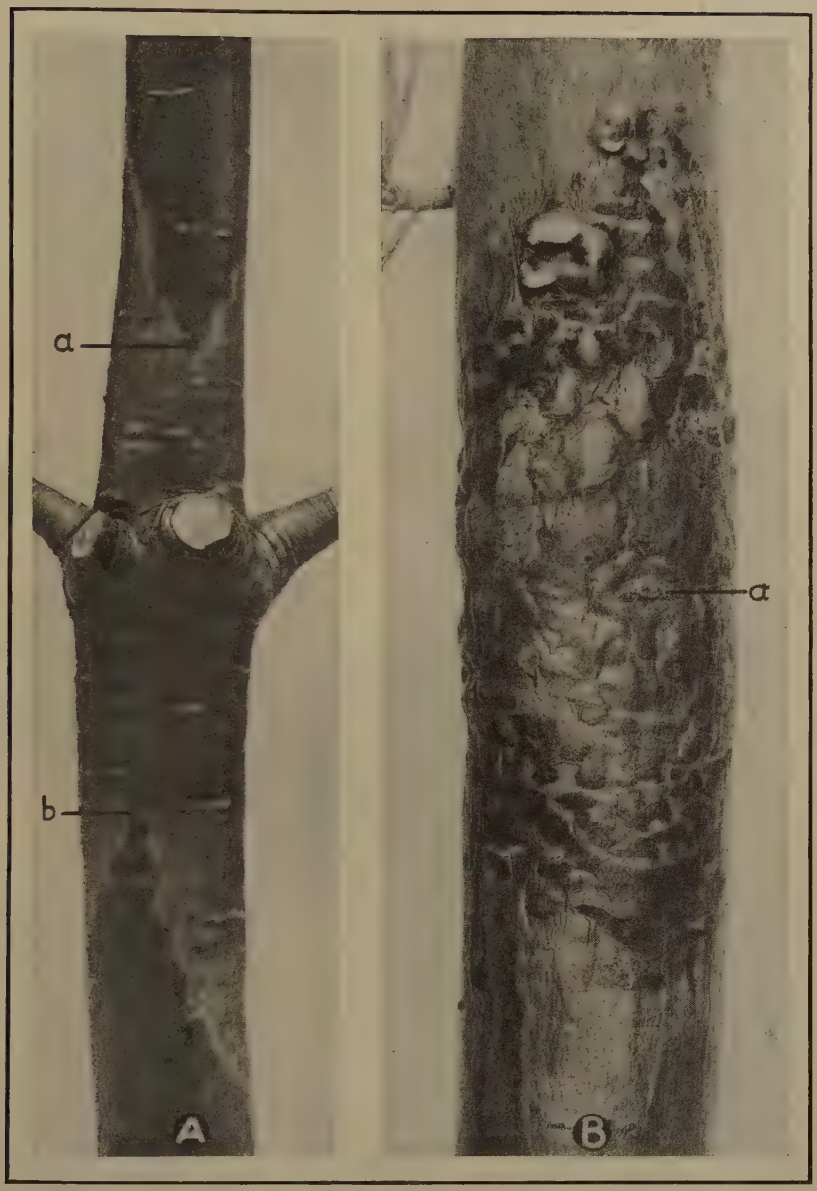
PLATE 48

Cronartium ribicola on *Pinus strobus:*

A.—This figure illustrates the etiolated condition of the bark in the case of a comparatively young nodal infection. The node lies between 4- and 5-year-old wood. The infection originated on the opposite side of the stem to that shown in the photograph. Note the irregular margin of the etiolated area. The mycelium, starting in opposite directions from the infection point, has now completely encircled the stem. The junction point of the encircling hyphæ is along the line *ab*. Natural size.

B.—This figure illustrates an internodal infection, somewhat older than that shown in figure A, in which the infection apparently originated at the base of the leaf fascicle (*a*). The darker patches on the pronounced canker area are pycnial spots. Æcia are formed under the bark all over the canker and are beginning to break out toward the upper end. Note the characteristic shape of the young canker. Natural size.


a
b
A
a
B

A
c
b
a
B
a
C

PLATE 49

Cronartium ribicola:

A.—The edge of a pycnium in section. Note the position of the pycnium with reference to the overlying periderm and underlying cells of the outer cortex. × 250.

B.—Part of the same section showing the general relation of the elements which go to make up the sorus and their relation to the host cells beneath. *a*, Contributing hyphæ; *b*, pseudoparenchyma layer; *c*, sporophores. Compare Pl. 58, A. × 1,050.

C.—Tangential section in the xylem, showing the cut end of a ray and the manner in which a haustorium (*a*) may rise from the hyphæ in the ray and enter the lumen of the tracheid. × 525.

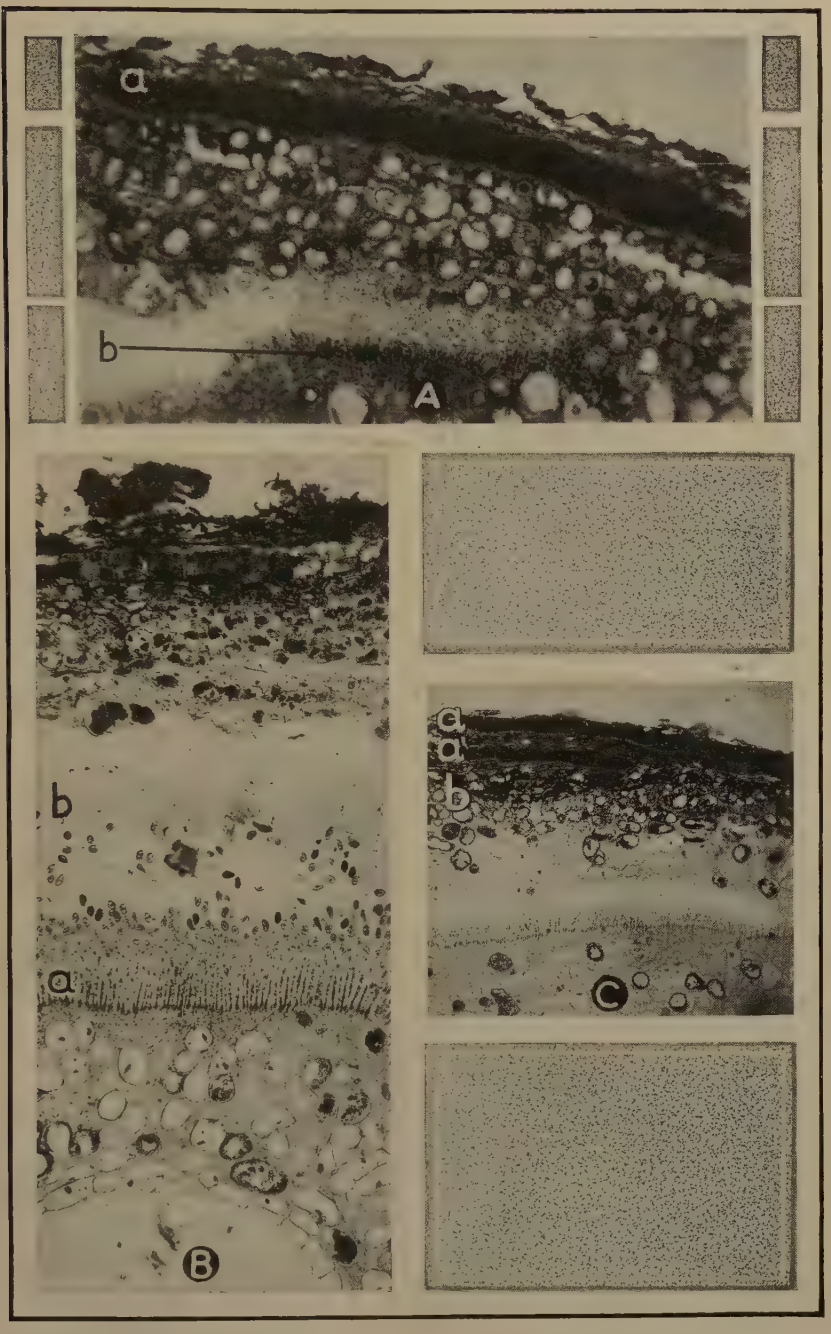
PLATE 50

Cronartium ribicola:

A.—A figure illustrating the relation of the pycnium (*a*), the heavy black line at the top, and young æcium, to the host tissue. The heavily stained cells (*b*) in the region of the young æcium are the fertile cells. Compare Plate 54, B. × 125.

B.—A section through a mature æcium, taken a little to one side of the break in the bark, to show the æciospore chains (*a*), the multilayered peridium (*b*), and the overlying host tissue. × 75.

C.—A similar section showing a double pycnial layer (a, a_1), and the location of the cork cambium (*b*) which cuts out the old pycnium. × 40.


a
b
A
b
a
B
a
a
b
C

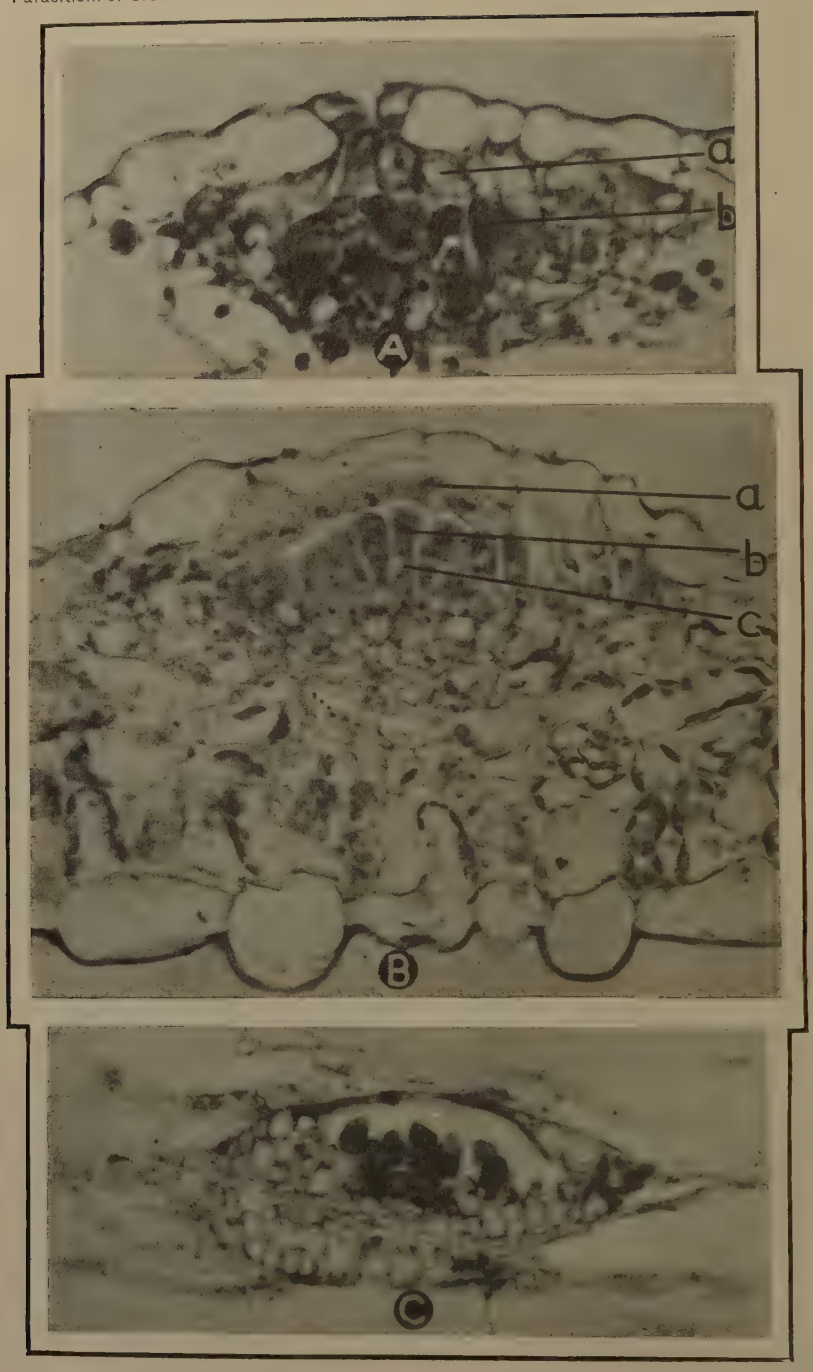

a
b
A
a
b
c
B
C

PLATE 51

Cronartium ribicola:

A.—A median section through a young uredinium forming in the space beneath a stoma. *a*, Peridial cells; *b*, young urediniospores. The photomicrograph illustrates a stage in the development of the uredinium midway between the stages shown in Plate 51, B, and 55, B. × 650.

B.—A section through the same uredinium as that shown in Plate 55, C, taken to one side of the break in the peridium, toward the edge of the sorus. *a*, Peridial cells; *b*, a young urediniospore; *c*, A stalk cell. × 525.

C.—An internal uredinium from the cortex of *Ribes hirtellum*. × 315.

PLATE 52

Cronartium ribicola:

A.—A section of a young telial column. Note the overlying peridial cells (*a*) and the row arrangement of the developing teliospores. The epidermal cells have been torn off. × 355.

B.—A later stage in the development of the telial column. Note the shape of the tip cells and the character of the parenchyma-like cells (*a*) surrounding the base of the young column (cf. Pl. 55, C). The cells near the base of the column are binucleate, while those at the tip are uninucleate. × 355.

C.—A longitudinal section of a mature column. × 250.

D.—Higher power view of the same section, showing the arrangement of the individual spores, and the size of the nuclei. × 1,050.

E.—A cross section of a small mature column. × 525.

a
A
a
B
C
D
E

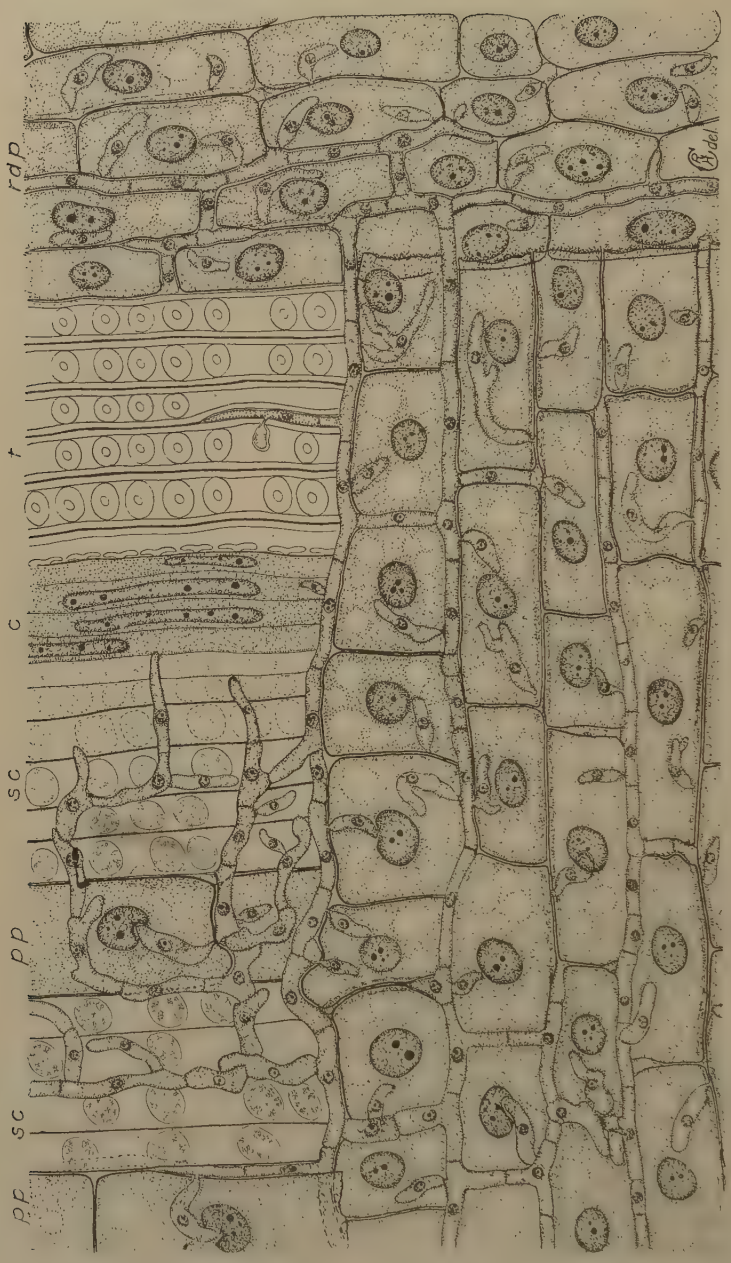

rdp
t
c
sc
pp
sc
pf
Bdel

PLATE 53

Cronartium ribicola:

A drawing to show the intimate relation of the mycelium of the parasite to the host cells. *pp*, Phloem parenchyma; *sc*, sieve cells; *c*, cambium cells; *t*, tracheids; *rdp*, resin-duct parenchyma. The number of haustoria represented as entering the cells in this drawing is not abnormally large. The drawing was made from two serial sections from the same area by means of a projection apparatus and a camera lucida and has been diagrammatized only so far as was necessary to bring some of the elements to a proper level for drawing. In a few cases nuclei, which were not present in the sections, were supplied for both host and parasite cells. Note the different shapes and sizes of the haustoria and the general character of the hyphæ lying between the cells. × 500.

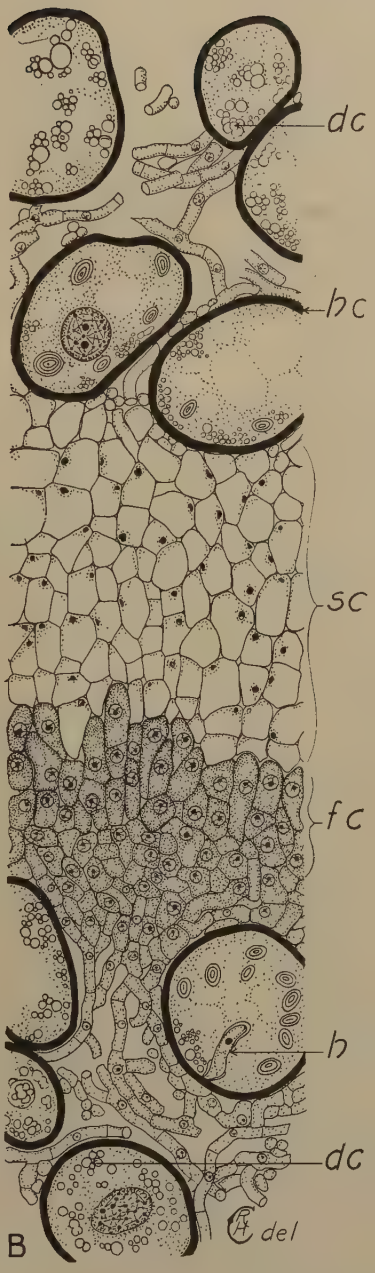
PLATE 54

Cronartium ribicola:

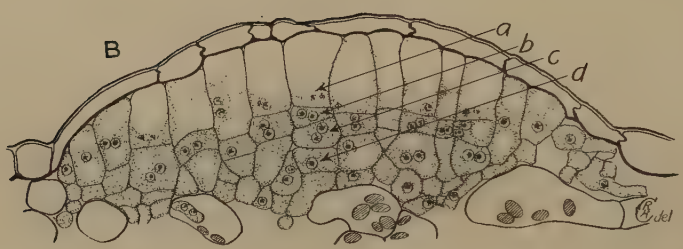
A.—A drawing of an infected 12-year-old main stem. The infection entered the main stem along the small branch, the stub of which is shown at the right of the figure. *a*, The advancing edge of the infection; *b*, the pycnial area. The black dots are the pycnial spots; *c*, the æcial area on which the bark is cracked and broken. In another season the æcial area would spread over the pycnial area (*b*), and the pycnial area would be advanced as far as the boundary (*a*) under normal conditions. The boundary (*a*) would be proportionately advanced also. The specimen from which the drawing was made was collected in August, 1917. × ½.

B.—Drawing of a section through part of a young æcium showing the relation of the fertile cells with their denser protoplasmic contents to the overlying sterile cells, in which the cytoplasm and nuclei have begun to go to pieces. The manner in which the adjacent host cells are forced apart by the fungus cells is also shown. *dc*, decomposition products in the host cells; *hc*, host cell wall; *sc*, sterile cells; *fc*, fertile cells; *h*, haustorium. The elliptical bodies in the host cells represent starch grains. × 400.

a
b
c
A
del


dc
hc
sc
fc
h
dc
B
del

A
del


B
a
b
c
d
del

C
d
e
f
g
a
b
c
del

PLATE 55

Cronartium ribicola:

A.—A drawing of a median section through a very young uredinium. Note that the fungus cells are closely compacted against the underside of the host epidermal cells, and that the binucleate cells adjacent to the epidermal cells are oriented with their long axis more or less perpendicular to the epidermis. The section from which the drawing was made was cut just to one side of a stoma. See text for further explanation. × 500.

B.—A drawing of a median section through a young uredinium, somewhat older than that illustrated in figure A. *a*, A peridial cell; *b*, a young urediniospore; *c*, a cell which is homologous to the stalk cell of an older sorus; *d*, a basal cell. See text for further explanation. × 500.

C.—A drawing of a median section through a small mature uredinium. *a*, A binucleate basal cell; *b*, a tetranucleate basal cell just before the urediniospore initial is cut off; *c*, a secondary urediniospore initial which has just been cut off from the basal cell; *d*, the urediniospore initial has divided into a urediniospore and a stalk cell; *e*, a mature urediniospore which is still connected with the basal cell by a collapsed stalk cell; *f*, crushed epidermal cells of the host; *g*, the bank of parenchyma-like cells which encircle the sorus. The cells of this group, which lie next to the epidermis, are homologous to the thickened peridial cells which overlie the greater part of the uredinium. Note the comparatively small opening in the peridium. The drawing has been slightly diagrammatized for the sake of completeness by combining some of the features from two sections. Compare Plate 51, B. × 500.

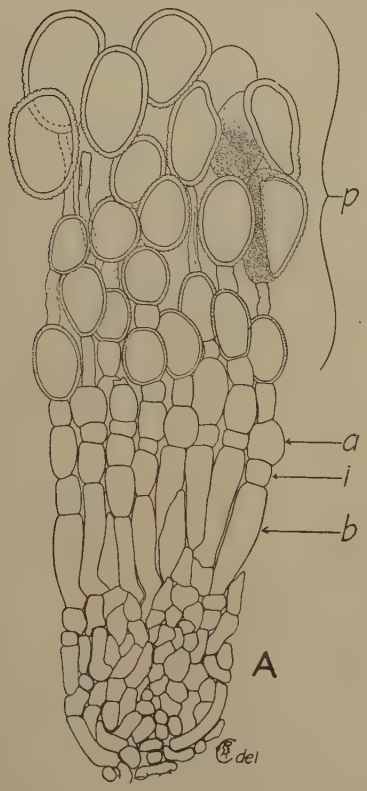
PLATE 56

Cronartium ribicola:

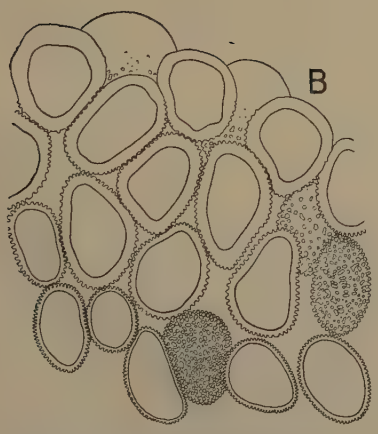
A.—A drawing of the cell relations near the edge of an æcium to illustrate the formation of the multilayered æcial peridium. *a*, A young æciospore; *i*, an intercalary cell; *b*, the basal cell of the chain; *p*, the potential æciospores which are being modified into peridial cells. Note the wall markings and the long degenerating intercalary cells. × 400.

B.—A drawing of a section through a mature peridium, taken from the same series as the photomicrograph in Plate 50, B. × 400.

C.—A drawing of a short mature telial column in which the teliospores (*a*) have germinated, producing promycelia and sporidia (*b*). See Plate 57 for details of the process. ×170. Drawn by Miss Minnie W. Taylor.


p
a
i
b
A
del

b
a
C


B

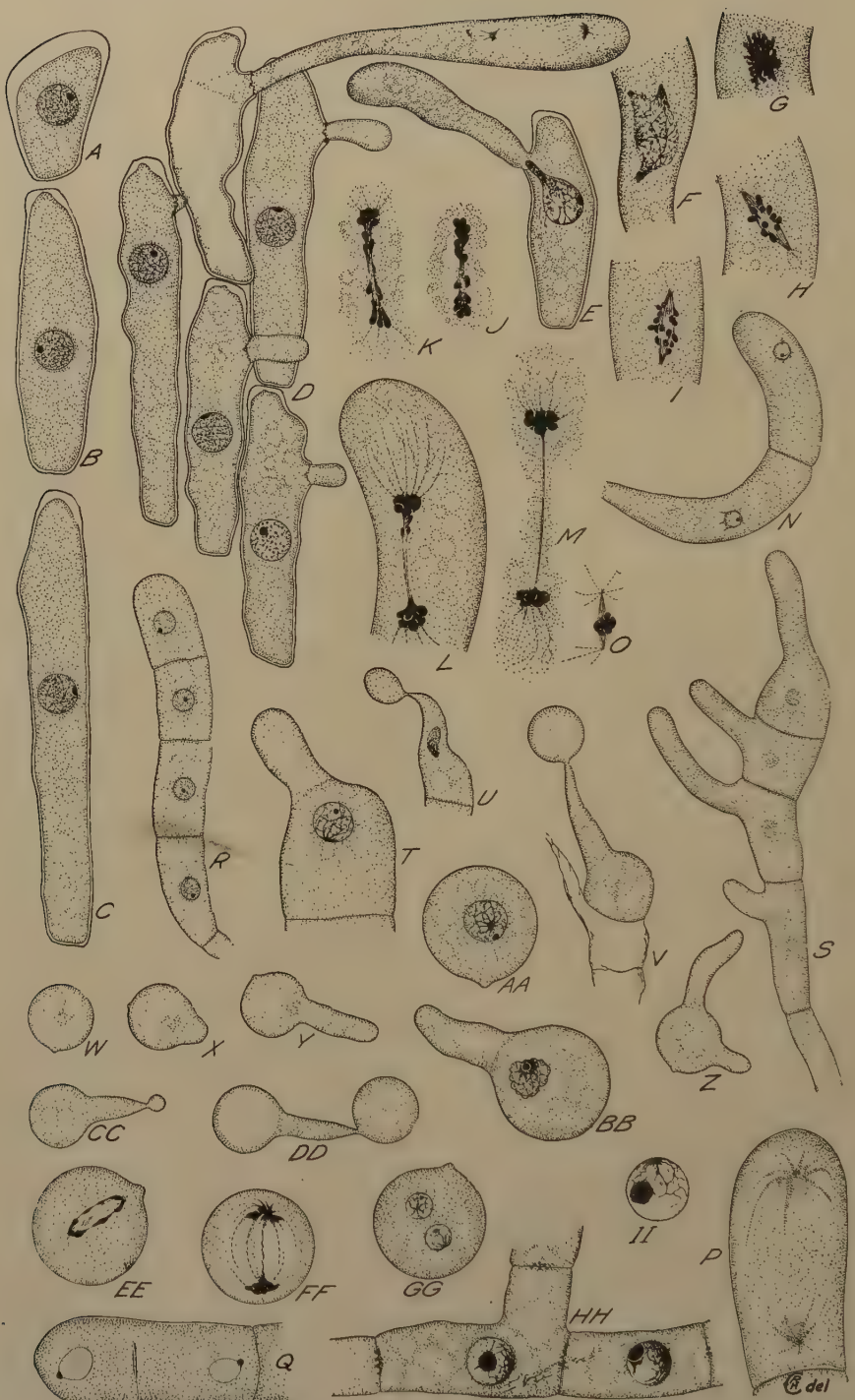

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PLATE 57

Cronartium ribicola:

A.—A mature teliospore from the tip of a column. × 850.

B.—A mature teliospore from the side of a column. × 850.

C.—A mature teliospore from the side of a column. × 850.

D.—Five germinating teliosporesfrom a longitudinal section of a column. The nucleus in the promycelium from the upper teliospore is in the telophase of the primary division. × 850.

E.—A germinating teliospore. The nucleus is about to pass through the germ pore into the promycelium. × 850.

F.—Early prophase of the primary division in the promycelium. × 1,700.

G.—Late prophase of the primary division in the promycelium. The chromatin is in the form of complex tangle. ×1,700.

H.—Early anaphase of the primary division in the promycelium. Eleven chromosomes are visible. ×1,700.

I.—Later anaphase than that shown in figure H. Sixteen chromosomes visible. × 1,700.

J, K.—Two anaphase stages of the primary division. The chromosomes are well advanced toward the poles. ×1,700.

L.—Late anaphase of the primary division. The chromosomes are condensing into deeply staining clumps, but the individuals are still discernible in some cases. ×1,700.

M.—End ot the anaphase of the primary division. The fibres connecting the two chromatin groups are drawn to a thin fading strand. Note the radiations in the cytoplams in this and the preceding figure. ×1,700.

N.—The 2-celled promycelium. ×850.

O.—Metaphase of the second division. × 1,700.

P.—Telophase of the second division. Peculiar cytoplasmic radiations run from the reorganizing nuclei. × 1,700.

Q.—The reorganizing nuclei after the second division. × 1,700.

R.—The completed promycelium. Each nucleus shows a small nucleolus and a definite centrosome. × 850.

S.—Surface view of a germinating promycelium. × 850.

T.—The tip cell of a germinating promycelium. The nucleus exhibits definite polarization. × 1,700.

U.—A little later stage than the last. The nucleus is passing into the sterigma on its way to the sporidium. × 850.

V.—Surface view of a tip cell of a promycelium bearing a sterigma and a nearly mature sporidium. × 850.

W.—A mature sporidium. The papilla marks the point of attachment of the sterigma. × 850.

X, Y, Z.—Steps in the germination of the sporidia. × 850.

AA.—Sectional view of a mature sporidium. The nucleus shows polarization. × 1,700.

BB.—Sectional view of a germinating sporidium. The nucleus appears to be moving toward the germ tube and preparing to divide. × 1,700.

CC, DD.—Two stages in the formation of secondary sporidia. × 850.

EE.—Midanaphase of the division of the sporidium nucleus. × 1,700.

FF.—Late anaphase of the same. × 1,700.

GG.—Sectional view of a binucleate sporidium. × 1,700.

HH.—Two cells from the vegetative mycelium in the pine. × 1,700.

II.—A definitely polarized nucleus from the vegetative mycelium in the pine, located just beneath the fertile layer of the young æcium. × 1,700.

PLATE 58

Cronartium ribicola:

A.—The elements of the pycnium. The cells at the base are almost empty. Above them are the short branching trunks which bear the sporophores. *a*, A sporophore; *b*, pycniospores in sectional view; *c*, the cytoplasm is constricted just beneath the spore; *d*, the nucleus is dividing. × 1,700.

B.—An active thin-walled haustorium from a pine host cell. × 1,700.

C, D, E.—Old haustoria. C and D have basal cuplike sheaths and tip sheaths. E is completely inclosed in a thick sheath. × 850.

F.—Telophase of division of one of the cells of the fertile layer to form a sterile cell. ×1,700.

G.—A newly formed sterile cell. × 1,700.

H.—A large polarized nucleus from the fertile layer. × 1,700.

I.—An æcial basal cell resulting from the fusion of two adjacent cells of the fertile layer. Compare the size of the nuclei with that of the nucleus shown in Pl. 57, II. × 1,700.

J.—A diagram of a basal cell resulting from the fusion of two cells from different levels. × 850.

K.—A diagram of a trinculeate irregular basal cell from the tip of which a trinucleate æciospore initial has been cut off. × 850.

L.—A diagram of part of an irregular compound fusion cell. × 850.

M.—A basal cell with the nuclei in early prophase. The centrosome in the left nucleus has apparently divided. *b*. An æciospore initial cell with the nuclei in midanaphase. × 1,700.

N.—Part of a basal cell. The nuclei in prophase. × 1,700.

O.—A later stage than the preceding. The spindle and centrosomes are just visible in the upper nucleus. × 1,700

P.—Metaphase of the division in the basal cell. A centrosome is evident at each of the poles of the spindles. The chromatin is condensed into chromosomes. The nucleoli have moved off and begun to fade. × 1,700.

Q.—Early anaphase, a little later than the stage in figure P. × 850.

R.—Midanaphase of the division. The chromosomes are moving toward the poles along the outside of the spindle. × 1,700.

S.—A later stage of anaphase than in figure R. × 1700.

T.—Final anaphase. The chromatin is condensing in two groups at each pole. × 1,700.

U.—Telophase. The two groups for each pole are still distinct. × 1,700.

V.—Telophase. The two groups at each pole have condensed to single masses. The wall which will separate the æciospore initial from the basal is beginning to form at *a*. × 1,700.

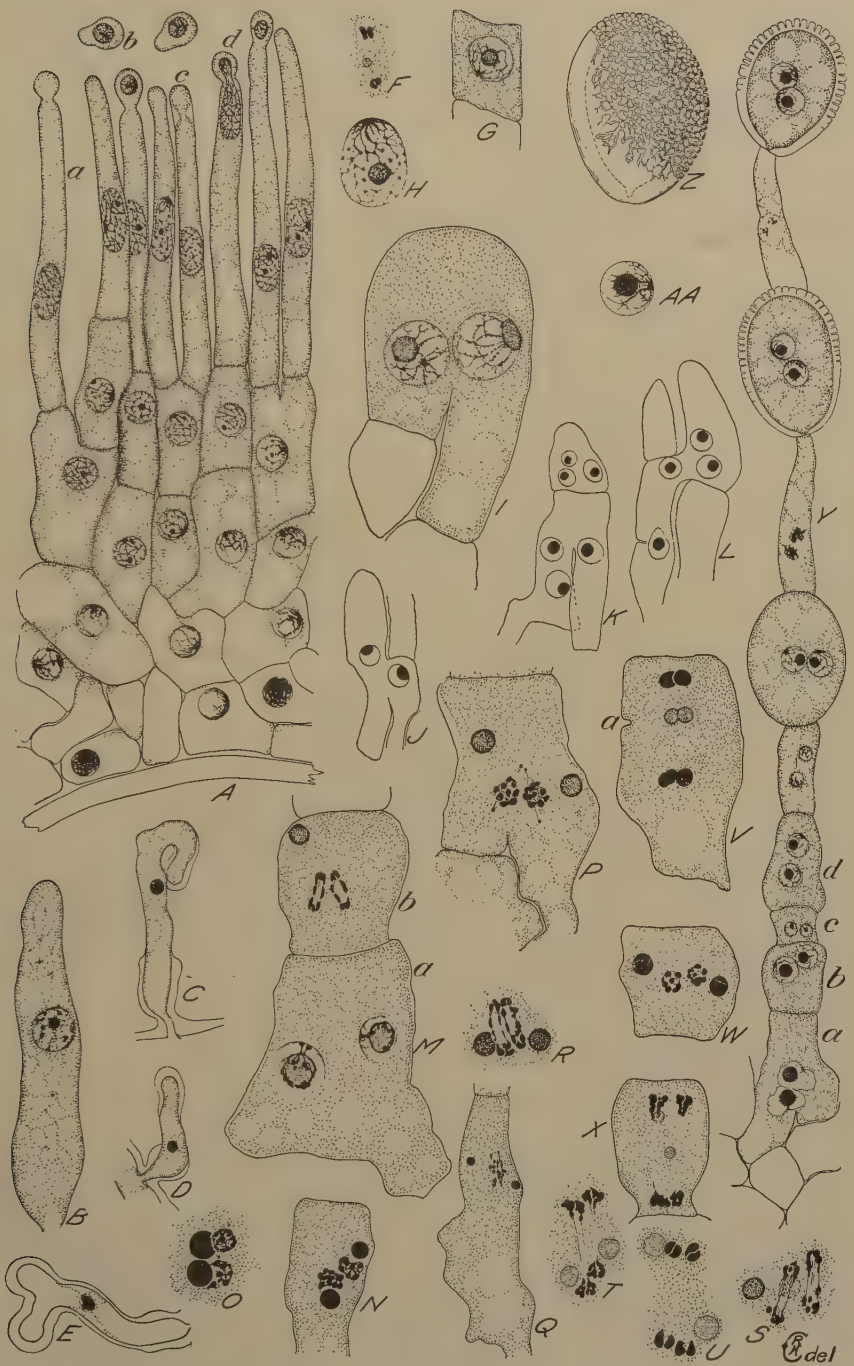
W.—Prophase of the division in the æciospore initial. × 1,700.

X.—Final telophase of the same. × 1,700.

Y.—An æciospore chain in section view. *a*, The basal cell; *b*, an æciospore initial, *c*, an intercalary cell; *d*, a young æciospore. The nuclei in the upper intercalary cells are degenerating. × 850.

Z.—A large mature æciospore in surface view. × 850.

AA.—A nucleus from a mature æciospore. × 1,700.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AA
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CC
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del

PLATE 59

Cronartium ribicola:

A.—Germinating æciospores. × 425.

B.—Sectional view of the æciospore wall showing the manner in which the germ tube is constricted. × 850.

C.—A binucleate cell from the mycelium in a leaf of *Ribes* sp. The nuclei are polarized as in the uninucleate mycelium. × 1,700.

D.—A binucleate haustorium from a host cell of *Ribes* sp. × 1,700.

E.—A uredinial basal cell. The nuclei are in prophase. × 1,700.

F.—Metaphase of the primary division in the basal cell. The chromosomes are visible as distinct units. × 1,700.

G.—Early anaphase of the same division. × 1,700.

H.—A later stage of the anaphase. × 1,700.

I.—Final anaphase. The two groups at each pole are not clearly distinct in this figure. × 1,700.

J.—A binucleate urediniospore initial. The nuclei are polarized. × 1,700.

K.—Metaphase or early anaphase of the division in the initial. × 1,700.

L.—Late anaphase in the initial. The chromatin is condensing in two groups at each pole. The second nucleus in the basal cell has been cut away. × 1,700.

M.—Late anaphase group from the initial, for comparison with figure L. × 1,700.

N.—A basal cell bearing a stalk cell surmounted by a nearly mature urediniospore (*a*), and a secondary urediniospore initial (*b*). The nuclei in the latter are in prophase. × 850.

O. Metaphase of the secondary division in the basal cell, preparatory to the formation of a secondary urediniospore initial. × 1,700.

P. A mature urediniospore. × 850.

Q. A germinating urediniospore in sectional view. × 850.

R.—A germinating urediniospore. × 425.

S.—A telial basal cell. Both nuclei show centrosomes and polarization phenomena. × 1,700.

T.—Metaphase of the division in the telial basal cell. × 1,700.

U.—Late anaphase of the same. × 1,700.

V.—Final anaphase of the same. × 1,700.

W.—Telophase of the same. × 1,700.

X.—A diagram of a telial unit column. Two young binucleate teliospores surmount the basal cell. × 850.

Y.—The two nuclei of the young teliospore just previous to fusion. Note the centrosomes. × 1,700.

Z. The two nuclei in the process of fusion. Two nucleoli and two centrosomes are still visible. × 1,700.

AA.—The large fusion nucleus. The chromatin is in heavy strands. × 1,700.

BB.—The fusion nucleus, slightly decreased in size. The chromatin strands are finer than in the preceding stage. × 1,700.

CC.—The mature fusion nucleus. The chromatin is in the form of granules. × 1,700.